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NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

ANNUAL REPORTS

DIVISION OF INTRAMURAL RESEARCH

October 1, 1994 to September 30, 1995

9

PROJECT NUMBERS

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

ACTIVE PROJECTS

Z01 DK 11000-02 ODIR
Z01 DK 11001-02 ODIR
Z01 DK 11500-02 ODIR

Z01 DK 13004-22 MRB
Z01 DK 13020-06 MRB

Z01 DK 15100-25 LCDB
Z01 DK 15102-35 LCDB
Z01 DK 15401-23 LCDB
Z01 DK 15404-11 LCDB
Z01 DK 15500-34 LCDB
Z01 DK 15503-14 LCDB
Z01 DK 15505-18 LCDB
Z01 DK 15506-11 LCDB
Z01 DK 15508-07 LCDB
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Z01 DK 17002-25 LBM
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Z01 DK 17009-10 LBM
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Z01 DK 22001-04 LCBG

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TERMINATED PROJECTS

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Z01 DK 69056-02 PECRB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 11000-02 ODIR

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physics of Ionic Channels and Other Proteins with Aqueous Cavities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: V.A. Parsegian, Guest Researcher, OD/DIR-NIDDK
Others: S.M. Beznukov, Visiting Scientist, OD/DIR-NIDDK

COOPERATING UNITS *(if any)*
NIST (J.J. Kasianowicz); Office of Naval Research Europe, London, UK(Dr. I. Vodyanoy); University of Maryland, College Park, MD (Dr. M. Colombini)

LAB/BRANCH
Division of Intramural Research

SECTION
Biophysics Unit

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have analyzed the signal transduction properties of voltage-dependent ion channels in the presence of an external electric noise source. Experiments were done with planar lipid bilayers modified by a well-studied channel-forming peptide, alamethicin. A current response to a small sine-wave voltage signal was measured at different levels of admixed "white" noise. We showed that addition of external noise to this system input induces a significant increase of signal component at its output. This noise-induced enhancement in signal transduction does not decrease the output signal-to-noise ration and shows some features of the 'stochastic resonance'.

As we showed previously using phospholipid species with different tendency to form non-lamellar structures, alamethicin channels are sensitive to the inherent stress of lipid packing within a membrane. Now, studying conductance probability distribution of alamethicin channels incorporated into a charged lipid membrane and varying charge per lipid molecule via pH of the membrane-bathing solution, we are able to relate quantatively the channel conformational equilibrium seen in its opening and closing statistics to the membrane pacing energetics.

An immediate consequence of these studies is the ability to observe how a single macromolecular unit is able to time-average the binding and dissociation of small molecular weight effectors.

This project has been terminated as of the 1995 fiscal year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 11001-02 ODIR

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sructure and Physical Properties of DNA and DNA-Protein Complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: D.C. Rau, Research Chemist, OD/DIR-NIDDK
Others: N.Yu. Sidorova, Guest Researcher, LSB-DCRT
V.A. Parsegian, Reseach Physicist, LSB-DCRT

COOPERATING UNITS *(if any)*
LPTB, NICHD (Dr. M. Garner); Towson State University, Towson, MD (Dr. R. Preisler); University of Nevada, Reno, NV (Dr. R. Harrington); LCB, NHLBI (Dr. E. Korn)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The transfer of the restriction nuclease EcoRI from nonspecific association with poly(dI-dC) to its recognition sequence is accompanied by the release of 110 waters that is independent of the solute used to set water activity for a wide range of solute sizes and chemical identities. As was first suggested by our previous measurements on the competitive binding of E. coli galactose operon repressor and is now confirmed by the more extensive measurements with EcoRI, the effect of solutes on the transfer reaction of protein from one DNA sequence to another is an ideally osmotic one. There is a well defined volume of water at the protein - DNA interface that is sterically sequestered from solute in the bulk solution. Water release can now be regarded as both a thermodynamic and a structural parameter, linking energetics and closeness of contact. We can now investigate in detail the relationship between water release and association energies using Cro protein and a series of DNA recognition sequences that span a range of binding constants. The measurement of forces between molecules in condensed arrays show a dominating contribution from water structuring. The previous measurements on the binding of gal repressor to different operator sequences show that a link between binding strength and structured water release does not exist for association reactions in dilute solution.

Changes in the structure and flexibility of Acanthamoeba myosin II minifilaments with ATP or ADP binding have been further characterized by electric birefringence. Minifilaments with bound ATP or ADP are about 20 fold more flexible than without nucleotide. This change in flexibility seems directly relevant to the force generating step in actin-myosin complexes. Comparison of signal amplitudes for native myosin II minifilaments and minifilaments constructed from truncated myosin (missing the S1 heads) indicates that flexing occurs at the LMM-HMM junction. Changes in the fast component relaxation kinetics indicates that nucleotide binding affects the structure of the S1-S2 junction or of the S1 heads as well as the flexibility of the LMM-HMM junction. The nature of the structural coupling of these two physically well separated regions is still unknown, but may involve the light chains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 11500-02 ODIR

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
DNA Repair and Replication in Xenopus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Eric Ackerman, Special Expert, OD, NIDDK
Others: Naoko Oda, Visiting Fellow, OD, NIDDK
Victoria Doseeva, Visiting Fellow, OD, NIDDK
Lilia Koriazova, Visiting Fellow, OD, NIDDK
Alexander Spoonde, Visiting Fellow, OD, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Director

SECTION

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Mayland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During early development Xenopus replicates its DNA nearly as fast as E. coli in log phase. Therefore, oocytes are an excellent source of proteins responsible for DNA repair and replication activity. We continued to characterize both DNA repair activity and single-strand to double-strand replication activity in Xenopus oocytes and our nuclear extracts. Pyrmidine dimers, (6,4) photoproducts and bulky chemical adducts undergo nearly complete repair, with no background synthesis on undamaged control DNA, in both microinjected oocytes and nuclear extracts. Unlike other extracts that repair <2% of the input damaged DNA, our extracts repair all of the damaged substrate and results in the extracts appear to accurately reflect events in living cells. Although the ss to ds replication activity is also very efficient both in oocytes and extracts, we demonstrated that surprisingly the presence of slight damage on a ss template completely suppresses the otherwise efficient replication reaction. This replication suppression may represent a new type of checkpoint for processing damage that either escaped G1 arrest or occurred after entry into S-phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13001-22 MRB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mathematical formulations and analysis relevant to experimental neurophysiology.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Rall	Senior Research Physicist	MRB, NIDDK
Others:	I. Segev	Fogarty Visiting Scientist (Summer)	MRB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13004-22 MRB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mathematical Description of Cellular Neuroelectric Signal Transmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John Rinzel	Chief	MRB, NIDDK
Others:	Yue-Xian Li	Visiting Fellow	MRB, NIDDK
	David Golomb	Visiting Fellow	MRB, NIDDK
	Bill Softky	IRTA Fellow	MRB, NIDDK
	Victoria Booth	IRTA Fellow	MRB, NIDDK
	Richard Bertram	IRTA Fellow	MRB, NIDDK
	Hagai Agmon-Snir	Human Frontiers Fellow	MRB, NIDDK
	Sharon Crook	Special Volunteer	MRB, NIDDK

COOPERATING UNITS (if any)
S Stojilkovic (ERRB, NICHD), JE Keizer (Univ Calif, Davis), X-J Wang (Univ Pittsburgh), Y Manor, I Segev, Y Yarom (Hebrew Univ), B Ermentrout (Univ Pittsburgh), C Carr (Univ MD), D Golomb (Technion, Haifa)

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS: 6.0	PROFESSIONAL: 5.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of electrical activity of neurons and other cell types. Among the topics of current interest are: (i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) propagation of action potentials along axons; (iii) stimulus-response and threshold properties for repetitive-firing of action potentials; (iv) complex bursting patterns of membrane potential oscillations which arise through endogeneous membrane properties and/or intercellular coupling.

Because qualitatively related mathematical or biophysical problems may arise in other context, e.g., chemical and biochemical oscillations, or e.g. excitation-secretion coupling, this project may consider models from such applications.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods drawn from both classical and modern applied mathematics. These methods may include finite difference or finite element numerical integration, bifurcation theory, perturbation methods, and nonlinear dynamical systems theory. One goal of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet physiologically reasonable, equations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13020-06 MRB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrical and Chemical Oscillations in Coupled Cell Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Arthur Sherman	Senior Staff Fellow	MRB, NIDDK
Others:	Richard Bertram	IRTA Fellow	MRB, NIDDK
	Paul Smolen	NRC Fellow	MRB, NIDDK
	Michael Mascagni	Guest Worker	MRB, NIDDK
	Manish Butte	Summer IRTA	MRB, NIDDK
	Tim Kiemel	Guest Worker	MRB, NIDDK

COOPERATING UNITS (if any)

C Stokes, L Xu (Dept of Chem Eng, Univ of Houston), B Soria (Dept de Fisiologia, Universidad de Alicante, Alicante, Spain) L Satin (Dept. of Pharmacology and Toxicology, Medical College of VA), LCBG, NIDDK, Bethesda, MD (I Atwater, E Rojas, D Mears); Synaptic Mechanisms Section, NINDS, Bethesda, MD (E Stanley)

LAB/BRANCH Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.6	PROFESSIONAL: 2	OTHER: .6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We use mathematical models to study the mechanisms of oscillatory electrical activity arising from ion channels in cell membranes and modulated by intracellular chemical processes. We are interested in both the behavior of single cells and the ways in which cells communicate and modify each other's behavior.

Our main application has been to the biophysical basis of insulin secretion in pancreatic beta-cells. We have examined bursting oscillations in membrane potential and the role of electrical coupling between cells in the islet of Langerhans. Long term goals are to understand how the membrane dynamics interact with intracellular events to regulate secretion and to generalize to other secretory cells and neurons.

Our primary tool is the numerical solution of ordinary and partial differential equations. We use analytical, geometrical, graphical, and numerical techniques from the mathematical theory of dynamical systems to help construct and interpret the models. Perturbation techniques are used to get analytical results in special cases.

We study both detailed biophysical models and simplified models which are more amenable to analysis. Such an approach aids the isolation of the essential or minimal mechanisms underlying phenomena, the search for general principles, and the application of concepts and analogies from other fields.

We see a role for our group as intermediaries between the mathematical and biological disciplines. This includes disseminating the insights of mathematical work to biologists in accessible language and alerting mathematicians and other theoreticians to new and challenging problems arising from biological issues.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-038

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15100-25 LCDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Nucleic Acid Interactions: Chromatin Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert T. Simpson	Laboratory Chief	LCDB, NIDDK
Others:	Hugh-George Patterton	Visiting Fellow	LCDB, NIDDK
	Yuko Tsukagoshi	Visiting Fellow	LCDB, NIDDK

COOPERATING UNITS (if any)

T. Richmond, ETH, Zurich, Switzerland (foreign)

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The involvement of chromatin structure in modulating DNA functions such as transcription, replication, recombination and repair has become increasingly apparent. We have shown that the placement of a nucleosome on a replication initiation site severely compromises replication function of the element. We have also shown that the MATα2p repressor precisely positions a nucleosome in the *STE6* gene adjacent to the α2 operator in a process that requires the histone H4 N-terminal tail. Although this nucleosome covers a downstream TATA box, shifting the TATA box to a position between two nucleosomes does not allow gene expression, suggesting that in this context transcriptional repression functions at an organizational level above the single nucleosome, namely a chromatin domain. We have shown that MATα2p organizes a unique gene-length domain comprised of a dinucleosome structural repeat with alternating short and long internucleosomal linkers. The disruption of other genes required for MATa2p-mediated repression causes disruption of this domain, even after reduction of the transcriptional activity of the derepressed gene to a fraction of the wildtype level, demonstrating the structural involvement of other non-histone proteins in the domain organization. We have investigated several aspects of the structure of this domain, and have shown that the domain can be structurally reproduced on a telomeric yeast plasmid. The domain appears to terminate at a position in the *STE6* gene that contains a perfect and several close matches to the ARS consensus sequence (ACS). This ACS region of the *STE6* gene acts as a functional replication origin in a yeast plasmid. Any mutation in this region that may interfere with origin recognition complex (ORC) protein binding results in the local disruption of the domain, demonstrating a structural involvement of the ACS region. We show that the polarity of the domain, which is asymmetrically propagated from the pseudo-symmetrical α2 operator, is not influenced by the direction of replication fork passage or downstream ORC binding. In isolation, the α2 operator with adjacent proximal sequences organizes flanking nucleosomes symmetrically in a telomeric plasmid.

We identified *HSP82* as a new member of the *Saccharomyces cerevisiae* early meiotic gene family and showed that basal expression of this gene is determined by the interaction of a promoter proximal heat shock element (HSE) with a nearby repressor element (URS1). We also identified an additional ancillary repressor element (ARE) that is required for efficient repression, and showed that the derepression of basal expression of *HSP82* utilizes an URS1-bound factor(s) that is genetically linked with the *UME6* gene. We proposed a mechanism of meiotic gene induction that proceeds via two distinct steps where the *IME1* gene product antagonizes URS1-mediated repression, followed by the stimulation of an HSE-bound factor by Ime2p, resulting in induction of *HSP82*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15102-35 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of a ribonuclease and its inhibitor from *Bacillus amyloliquefaciens*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert W. Hartley	Research Physicist	LCDB, NIDDK
	Milan Jucovic	Visiting Fellow	LCDB, NIDDK
	Daniela Krajcikova	Special Volunteer	LCDB, NIDDK
	Lilia Znamenskaya	Special Volunteer	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. Y. Mauguen, Centre Pharmaceutique, Univ de Paris-Sud; Dr. J. Sevcik, Slovak Acad Sci, Bratislava; Drs. A. Makarov and S. Deyev, Russian Acad Sci, Moscow; Dr. I. Leschinskaya, Kazan University, Kazan, Russia; Dr. C.N. Pace, Dept Med Biochem, Texas A&M, College

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two proteins, barnase, the extracellular ribonuclease of *Bacillus amyloliquefaciens*, and barstar, its intracellular inhibitor, are used as a model system for the study of protein folding and protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes. Recombinant DNA techniques are being applied with three major aims: (1) to facilitate production of wild type and mutant proteins; (2) to examine the structural and control sequences of the genes; and (3) to make specific changes in the sequences to test theories of folding and to probe the barnase-barstar interaction. Both proteins can now be obtained from recombinant genes in *E. coli* where expression of barstar counters the lethal effect of barnase expression. The structures of both proteins and their complex are known. A fast and relatively precise assay has allowed the development of techniques for studying the kinetics and stability of complex formation. The barnase-barstar interface is being explored by an in vivo technique which selects suppressor mutations which rescue the system from lethal mutations that interfere with barnase inhibition. For example, barnase(H102K) with wild type barstar is conditionally lethal in the appropriate vector. Several mutations at Tyr29 and Tyr30 of barstar allow greater production of the mutant barnase and bind more tightly to the mutant barnase in vitro. Structural studies of several such mutant combinations are under way.

Barstar also inhibits a group of RNases from *Streptomyces* strains. These enzymes are distantly related to barnase with a sequence identity of only 25%. Among themselves, identity ranges from 40% to 70%. We have the genes for four such enzymes expressed in *E. coli* with the aid of the barstar gene. For three of these, yields are already in the 50-100 mg/ml range. The structures of two of the enzymes are already known from work on nonrecombinant material. A collaborative effort is under way to apply this group, along with barnase and its closer relatives, to structural and folding studies, alone and in combination with barstar. Isolation and cloning of putative homologs of barstar are being pursued.

Work elsewhere, in which the barnase gene becomes a tissue-specific killer when attached to eukaryotic promoters, has aroused considerable interest in its use in developmental studies and as the key to a variety of anti-viral strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15401-23 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis and Transport of Lipoprotein and Hepatic Lipases in Cells and Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert O. Scow	Scientist Emeritus	LCDB, NIDDK
Others:	Charles J. Schultz	IRTA Fellow	LCDB, NIDDK
	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
	Albert E. Spaeth	Chemist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. J.-W. Park, Dept. of Biochem., Chonbuk Natl. Univ. Med. School, Chonju, Chonbuk, Republic of Korea; Dr. T. Takahashi, Dept. of Oral Anatomy, Kanagawa Dental College, Yokosuka, Kanagawa, Japan

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.0	OTHER: 0.5

CHECK APPROPRIATE BOXES
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipoprotein lipase (LPL) is required for uptake of triacylglycerol by tissues from plasma lipoproteins. It is synthesized in parenchymal cells and transferred to capillaries where it acts. Active LPL is a noncovalent homodimer of N-linked glycosylated subunits and has a high affinity for heparin. The relation between glycosylation, dimerization, affinity for heparin, and activity of LPL was studied in cultured brown adipocytes of newborn mice. It was found that normal cells contained several forms of glycosylated LPL. The most prevalent form was dimeric with high heparin affinity and catalytic activity. Less prevalent forms were monomeric, dimeric, and higher oligomeric, all with low heparin affinity and no activity. Findings in cells treated with 1-deoxymannojirimycin demonstrated that dimerization, high heparin affinity and activity of LPL did not require processing of oligosaccharides in Golgi. Cells treated with tunicamycin synthesized unglycosylated LPL which was dimeric, had low heparin affinity, and was inactive. Blockade of glucosidase I in endoplasmic reticulum (ER) of normal cells with castanospermine (CST) had no effect on dimerization or acquisition of high heparin affinity. However, it prevented processing of LPL to an active form. Hence, one-fourth of LPL in CST-treated cells was dimeric and had high heparin affinity, but was inactive. Earlier studies showed that CST caused retention of LPL in ER. Adipocytes cultured from *cld/cld* mice synthesized fully glycosylated LPL which was dimerized, had high heparin affinity, and was trimmed of sugar residues in ER, but the lipase was inactive and retained in ER. Translocation of Golgi components to ER by brefeldin A resulted in synthesis of active LPL and processing of one oligosaccharide per subunit to endo H-resistance in CST-treated and *cld/cld* cells. The findings indicate that LPL synthesized in *cld/cld* cells is capable of being processed to active lipase and the enzymes needed for activation of LPL are present in Golgi, but the cells are unable to transport LPL to Golgi. We conclude that high heparin affinity of LPL requires glycosylation and dimerization but not trimming of oligoglycans in ER, whereas catalytic activity of the lipase requires high heparin affinity, trimming in ER, and some modification other than oligoglycan processing by Golgi, in addition to glycosylation and dimerization. Furthermore, glycosylation, dimerization and acquistion of high heparin affinity occur in ER, while activation occurs in Golgi. Schematic models were developed for the four LPL dimers found in cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15404-11 LCDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructural Immunocytochemistry of Lipid Metabolism in Cells and Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
Others:	Robert O. Scow	Chief, Endocrinology Sect.	LCDB, NIDDK
	Nancy K. Dwyer	Biologist	LCDB, NIDDK
	Edward Neufeld	IRTA, Post-Doctoral Fellow	LCDB, NIDDK
	Therese Barber	IRTA, Pre-Doctoral Fellow	LCDB, NIDDK
	Hailan Tang	Special Volunteer	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Peter Pentchev. Dev Metab. Neurol Branch, NINCDS, NIH; Dr. Constantine Londos, Lab Cell Develop Biol, NIDDK, NIH; Dr. Richard E. Pagano, Mayo Clinic, Rochester, MN; Dr. R.M. Evans, University of Colorado, Denver, CO.

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to Z01 DK 60003-01 LCBB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15500-34 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Large-Scale Production and Purification of Compounds with Biological Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph Shiloach	Research Chemist	LCDB, NIDDK
Others:	Jeanne B. Kaufman	Biologist	LCDB, NIDDK
	Min-Ying Wang	Visiting Fellow	LCDB, NIDDK
	Loc B. Trinh	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Biotechnology Unit, Office of the Chief

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOXES

☐ (a) Human ☐ (b) Human ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biotechnology Unit is responsible for the production and purification of biological material. The Unit undertakes a wide array of biological production tasks such as large-scale growth of procaryotic cells and eucaryotic cells, production of various compounds from microorganisms and eucaryotic dells, and purification of biological compounds especially proteins. These products, not available from commercial sources, are needed for clinical and structural studies.

The Unit is capable of handling various production problems associated with widely different topics. The Unit performs this task by implementing both physiological and technical approaches. Efficient process development and production is possible to perform since the Unit possesses two integrated elements: fully operational multi-purpose pilot production facility and the capability of conducting research and development.

During the last 12 months the Unit performed 200 different large-scale preparations.

The Unit continues its effort to produce large amounts of proteins needed for various clinical use: S antigen from bovine retina, currently in the second phase of the clinical trials, and several modified bacterial protein toxins needed for the preparation of conjugated vaccines. The facility continues to prepare large quantities of various mammalian cells and start a project for the production of a transmission-blocking vaccine for malaria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15503-14 LCDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Developmental Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan R. Kimmel	Research Chemist	LCDB, NIDDK
Others:	John L. Medabalimi	Senior Staff Fellow	LCDB, NIDDK
	Gail Ginsberg	Biologist	LCDB, NIDDK
	Jasmine Gruia-Gray	Visiting Fellow	LCDB, NIDDK
	Delwood Richardson	Senior Staff Fellow	LCDB, NIDDK
	Kelley Rogers	IRTA Fellow	LCDB, NIDDK
	Peter Balint-Kurti	IRTA Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Constantine Londos, Membrane Regulation Section, LCDB, NIDDK

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Molecular Mechanisms of Development Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	7.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dictyostelium development is initially characterized by the appearance of non-terminally differentiated prestalk and prespore cells. At culmination of development these cells, respectively, become the terminally differentiated vacuolized stalk and heat resistant spore cells. G protein/receptor response to secreted, extracellular cAMP is critical for these developmental events. We have now demonstrated that cAMP signal response for prestalk and prespore development differ, and that receptor function can ultimately act through both cell autonomous and non-autonomous pathways. In particular, stimulation of cAMP receptor, subtype 4 (CAR4) activates prestalk differentiation through an intracellular signalling cascade. Similarly, stimulation of CAR1 activates prespore differentiation but, CAR4, also acting in a cell autonomous manner in prespore cells, appears to be linked to an inhibitory pathway. In addition, a population of cells that express CAR4 also secretes a positive regulator of prespore differentiation. CAR4 is not required for response to this secondary activator. The rZIP protein, that potentially acts downstream of CAR4 in prespore regulation, fractionates with nuclei and is capable of complex protein interaction through several specific domains. rZIP appears to sense and regulate a prespore signal perhaps mediated via the cAMP-dependent protein kinase (PKA). We have also identified a mutation that appears to be required for the differentiation of a population of prestalk cells that are responsible for activating the PKA gradient through the prespore region. Mutant cells differentiate into prespore cells poorly and do not form spores.

The lipid droplet associated protein perilipin may play an important role in the differentiation of adipocytes. Preadipocyte cells that are blocked in their ability to accumulate perilipin arrest early after developmental stimulation. In contrast, already differentiated steroidogenic cells that lack perilipin may metabolize cholesterol ester droplets normally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15505-18 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Adipocyte Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Constantine Londos	Research Chemist	LCDB, NIDDK
Others:	Christina Rondinone	Visiting Fellow	LCDB, NIDDK
	Daniel M. Levin	Special Volunteer	LCDB, NIDDK
	Diane L. Servetnick	Biologist	LCDB, NIDDK
	Dawn L. Brasaemle	Senior Staff Fellow	LCDB, NIDDK
	Charles J. Schultz	IRTA Fellow	LCDB, NIDDK
	Anita Goldhar	Special Volunteer	LCDB, NIDDK

COOPERATING UNITS *(if any)*
A.R. Kimmel, E.J. Blanchette-Mackie, J. Gruia-Gray, LCDB, NIDDK; J. Wolff, LBP, NIDDK; R.P. Nordan, DCT, NCI; A.S. Greenberg, Tufts University

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 5.0	PROFESSIONAL: 4.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The perilipins are a family of proteins, first identified in this laboratory, that are expressed only in adipocytes and in steroidogenic cells, where they coat the surfaces of intracellular neutral lipid droplets. The hydrolysis of lipids in these cells is initiated by protein kinase A-mediated phosphorylation hormone-sensitive lipase (HSL) in adipocytes and the closely related cholesteryl esterase in steroidogenic cells. Protein kinase A also polyphosphorylates the perilipins, which combined with their tissue distribution and subcellular location strongly imply a role for these proteins in the hyrolysis of lipids by the HSL/CEase class of enzymes. By confocal immunocytochemistry, we find that acute stimulation of lipolysis in adipocytes leads to a dispersion of the perilipin from the surface of larger lipid storage droplets. Concomitantly, HSL translocates from the cytoplasm to the surface of the lipid droplets. The dispersion of perilipin and translocation of HSL are reversed upon removal of the lipolytic stimulus. These data suggest that the perilipins act as a barrier to protect lipids from inappropriate hydrolysis by HSL.

The appearance of perilipin in differentiating adipocytes is closely linked temporally to the deposition of lipid, and previously we found that fatty acid synthesis was required for perilipin expression. We have studied the effects of lipid loading on perilipins in steroidogenic Y-1 adrenal cortical cells, which are more suitable for examining the effect of exogenous lipids than adipocytes, . First, exogenous fatty acids increase the expression of the mRNA for the A but not the C isoform of perilipin, suggesting that perilipin A may be important in the early stages of lipid accretion. Second, increased lipid depositions upon lipid loading of cells dramatically increases perilipins by stabilizing the proteins. Thus, lipids regulate the content of perilipin by two modes.

The cytokine, interleukin-6 (IL-6) has been implicated in several types of disease-associated cachexia. Previously, we found that IL-6 increases the tyrosine phosphorylation MAP kinases, the transcription factor, STAT3, plus several other proteins in 3T3-L1 adipocytes. We have identified two of these proteins as JAK-1 and JAK-2, members of the Janus kinase family that phosphorylate the STATs; this cytokine receptor/JAK/STAT scheme is thought to constitute a "short circuit" for cytokine activation of gene transcription. However, the serine/threonine kinase inhibitor, H-7, inhibits IL-6 induced tyrosine phosphorylation of the JAKs without affecting STAT3 tyrosine phosphorylation. Moreover, H-7 inhibited the tyrosine phosphorylation of nearly all other proteins by IL-6. These data point to an upstream serine/threonine kinase early in IL-6 signalling, and raise questions about the role of the JAKs in tyrosine phosphorylation of STATs in adipocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15506-11 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Gene Expression in Mammalian Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jurrien Dean	Section Chief	LCDB, NIDDK
Others:	Li-Fang Liang	Staff Fellow	LCDB, NIDDK
	Ann Ginsberg	Staff Fellow	LCDB, NIDDK
	Robert McIsaac	IRTA Fellow	LCDB, NIDDK
	Tracy Rankin	IRTA Fellow	LCDB, NIDDK
	Philip Castle	IRTA Fellow	LCDB, NIDDK
	Gail Osawa	Chemist, GS-11	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Zhi-Bin Tong and Larry Nelson, DEB, NICHD; Eric Lee and Heiner Westphal, LMGD, NICHD

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Mammalian Developmental Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mouse ovary serves as a paradigm for investigating the developmental biology of mammalian gonadogenesis, oogenesis, and fertilization.

Gonadogenesis: Mouse gestation occurs over 20 days. Germ cells, first detected in the developing embryo 7.5 days post coitus (dpc), migrate from the allantois to the genital ridge by 12.5 dpc. In XX females, the primitive gonad then differentiates into an ovary. Poly(A^+) was purified from female and male genital ridges isolated 12-13 dpc. cDNA libraries have been made for each sex and subtractive cloning techniques are being used to identify female-specific gene products involved in early sex determination and gonadogenesis.

Oogenesis: The zona pellucida, composed of three glycoproteins (ZP1, ZP2, ZP3), is an ovary-specific extracellular matrix that surrounds growing oocytes. We have cloned the three mouse and human genes and have demonstrated in mice that their coordinate expression is restricted to oocytes. Approximately 200bp upstream of the start of transcription of each of the three genes is an E-box that is necessary and sufficient for high level reporter gene expression in oocytes. By gel mobility shift assays, we have identified a heterodimer composed of basic helix-loop-helix transcription factors (the ubiquitously expressed E12 and ZAP-1, Zona Activating Protein) that bind to this E-box. These studies provide insight into the molecular basis for oocyte-specific gene expression and identify early markers of oocyte growth and differentiation.

Fertilization: The three zona proteins are secreted and form an extracellular matrix that mediates the relatively species-specific events of fertilization. To assess protein-protein interactions in the assemblage of the zona matrix, we have established tissue culture lines that express each of the zona proteins and have developed transgenic mice that either lack a particular zona protein or express the human homologue. Breeding studies and *in vitro* sperm-egg binding studies are being used to investigate the role of individual zona proteins in the assemblage of the zona matrix and to determine the molecular basis for the specificity of fertilization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15508-07 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromatin Structure in Regulation of Mammalian Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ann Dean	Research Chemist	LCDB, NIDDK
Others:	Qi-Hui Gong	Visiting Scientist	LCDB, NIDDK
	Jennifer McDowell	IRTA Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Don M. Wojchowski, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, State College, PA

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.75	PROFESSIONAL: 2.75	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The embryonic epsilon-globin gene is the first member of the beta-globin family of genes to be expressed during development. Expression of the beta-like-globin genes is under the control of the beta-globin locus control region (LCR), encompassing erythroid specific DNase I hypersensitive sites (HS) 5-18 kilobases upstream of the epsilon-globin gene. The LCR has long range effects on the chromatin structure of the beta-globin locus, and it activates transcription of the different globin gene promoters as the genes are expressed sequentially during development. Much of our current understanding of the process of gene activation has been gained from *in vitro* studies where DNA is not assembled with histones and other chromosomal proteins as it is in the nucleus. To study the activation of a gene in its natural context of chromatin *in vivo* we have used minichromosomes containing the human epsilon-globin gene and portions of the beta-globin LCR enhancer. Expression of the minichromosomal epsilon-globin gene in K562 human erythroid cells is completely dependent on the presence of the beta-globin LCR *in cis*. Using restriction endonuclease access, and indirect end labelling experiments, we have observed that the promoter and 5' proximal structural regions of the minichromosomal epsilon-globin gene are covered by an array of positioned nucleosomes. Both types of studies suggest alteration or loss of a single promoter-proximal nucleosome upon activation of the gene for transcription. Regulated expression of the epsilon-globin gene on the minichromosomes offers a means too study at high resolution, the promoter-enhancer interaction of an activated gene assembled *in vivo* into chromatin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 15509-04 LCDB

PERIOD COVERED
October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Polyprotein Processing in Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John L. Medabalimi	Senior Staff Fellow	LCDB, NIDDK
	Ewald M. Wondrak	Visiting Associate	LCDB, NIDDK
	Alan R. Kimmel	Research Chemist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. N.T. Nashed, Section on Oxidative Mechanisms, LBC, NIDDK; Dr. Paul Wingfield, Protein Expression Laboratory, NIH

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Molecular Mechanisms of Development Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have expressed in *E.coli* and purified a native protease precursor (TF-PR) of the human immunodeficiency virus, type 1, that contains the protease (PR) coding sequence linked to 60 residues of the native flanking trans-frame (TF; otherwise termed p6*) region at the N-terminus and investigated the kinetics of its maturation to release the mature protease. Upon renaturation, the TF-PR which possesses very low enzymatic activity undergoes time dependent autoprocessing to release the mature protease concomitant with an increase in enzymatic activity. Initial rates monitored by following the increase in enzymatic activity are linearly dependent on protein concentration, indicating that the N-terminal cleavage is first-order in protein concentration. The rate of disappearance of TF-PR and that of the appearance of the mature protease and enzymatic activity are identical; $k = 0.13 \pm 0.01\ \mathrm{min}^{-1}$, in 50 mM acetate at pH 4.8. A complete pH-rate profile for the autoprocessing reaction showed that cleavage of the N-terminal trans-frame region to release the mature protease is dependent on the ionization of groups with pKa's of 4.9 and 5.1. The TF-PR fusion protein is *ca.* 8-fold more sensitive to urea denaturation as compared to the mature protease suggesting that the presence of the TF sequence linked to the protease domain lowers the stability of TF-PR dimer. Kinetics of the autoprocessing of the TF-PR is consistent with previously published results for the autoprocessing of the model precursor MBP-ΔTF-PR-ΔPol (*Louis et al. Proc. Natl Acad. Sci USA 1994, 91, 7970-7974*) providing direct evidence that the intramolecular cleavage that occurs at the N-terminus of protease is independent of the length of sequence flanking the N-terminus of the protease.

We prove the presence of a protease cleavage site 8 residues downstream from the mature nucleocapsid protein in the transframe region of the *Gag-Pol* polyprotein using polyprotein and synthetic peptides as substrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 17001-29 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Role of Carbohydrate Moiety of Glycoproteins in Cellular Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ashwell	Institute Scholar	LBM, NIDDK
Others:	W. Berlin	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work carried out in this laboratory during the fiscal year 1995 can be divided into four separate areas including (a) initiation of studies on the role of the neuronal cell adhesion molecule (NCAM) in thyroid pathology (b) cloning of the ASGP receptor (c) synthesis of a novel histochmical substrate and (d) synthesis of a new chloride channel blocker. Since this laboratory will be closed as of September 1995, no outline of future studies is included.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 17002-25 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Enzymatic Basis of Detoxication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Jakoby	Chief, LBM	LBM, NIDDK
Others:	David Marshall	Visiting Fellow	LBM, NIDDK
	Y.-L. Yang	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH	Laboratory of Biochemistry and Metabolism
SECTION	Section on Enzymes and Cellular Biochemistry
INSTITUTE AND LOCATION	NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of a program in which the enzymes that participate in the process of detoxication are being examined as to their catalytic mechanism, attention is being concentrated on a sulfotransferase (aryl sulfotransferase IV). The enzyme catalyzes the transfer of the sulfuryl group from 3-phosphoadenosine 5-phosphosulfate (PAPS) to a very broad range of phenols; the products are 3-phosphoadenosine 5-phosphate (PAP) and the corresponding phenol sulfate. This laboratory has cloned the sulfotransferase from rat liver mRNA and can express it in very large quantities in Escherichia coli. In purifying the recombinant enzyme, however, two distinct forms of the sulfotransferase were physically separable. Labeled as forms A and B, both catalyzed the reverse reaction, i.e. the formation of free phenol from the phenol sulfate and PAP, but only form B could catalyze the physiological reaction. Further investigation revealed that form A had PAP bound to it so tightly that it would not allow the physiological donor, PAPS, to bind to the enzyme.

The data suggested that protein cysteine plays a role in the formation of the two forms since PAP can be released by oxidation of protein thiols. The sulfotransferase consists of two subunits of identical amino acid constitution, each of which has five cysteine residues. Mutants have now been obtained for each cysteine in which that residue has been converted to serine. At present, the role of each of the cysteines in the folding of the protein and the binding of PAP is being dissected. Control of the oxidation state of the sulfotransferase and enzyme activity, can be regulated by the redox potential of the enzyme solution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 17003-28 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Polysaccharides in Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Cabib	Senior Research Chemist	LBM, NIDDK
Others:	Richard Ford	IRTA	LBM, NIDDK
	Tomas Drgon	Visiting Fellow	LBM, NIDDK
	Jana Drgonova	Special Volunteer	LBM, NIDDK
	Roman Kollar	Special Volunteer	LBM, NIDDK

COOPERATING UNITS (if any)
Departamento de Microbiologia, University of Salamanca, Spain (Angel Duran); Slovak Academy of Sciences, Institute of Chemistry, Bratislava, Slovakia (Vladimir Farkas); CRADA, American Cyanamid, Princeton, NJ

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Morphogenesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4	PROFESSIONAL: 4	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Systematic deletions of the yeast CHS1 and CHS2 genes showed that the nonhomologous amino-terminal portions of the corresponding proteins, chitin synthetases 1 and 2, are not essential for either enzymatic activity or function. However, small further deletions into the homologous domain, either from the amino or carboxyl terminus, were deleterious to activity and physiological function. A continuing investigation into the architecture of the yeast cell wall led to the isolation of a fraction containing chitin, mannoprotein, ß(1- 6) and ß(1- 3)glucan, the structure of which is under study. Preparations of the GTP-binding protein that regulates ß(1- 3)glucan synthetase have been scaled up to isolate sufficient amounts of the protein for further study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 17008-12 LBM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Nuclear Envelope in Intracellular Protein Sorting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J.A. Hanover	Research Chemist	LBM, NIDDK
Others:	S. Bailer	Visiting Fellow	LBM, NIDDK
	W. Lubas	Research Associate	LBM, NIDDK
	M. Miller	IRTA	LBM, NIDDK
	T. Sweitzer	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Cell Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01-DK 60000-01 LCBB.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 17009-10 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic approaches to understanding mammary gland development and function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Hennighausen	Research Chemist	LBM, NIDDK
Others:	R.A. McKnight	Staff Fellow	LBM, NIDDK
	Gertraud Robinson	Senior Staff Fellow	LBM, NIDDK
	Kathrin Heermeier	Visiting Fellow	LBM, NIDDK
	Xiuwen Liu	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)
US Department of Agriculture (Robert Wall), Priscilla Furth (U. of Maryland Medical School), Gilbert Smith (NCI), Glenn Merlino (NCI), Andreas Zimmer (NIMH), Peter Gruss (Max-Planck-Institute, Gottingen, Germany), Avi Shamay (The Volcani Institute, Rehovet), Jeff Pollard (Albert-Einstein College, Bronx), Derek LeRoith (NIDDK)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Develpmental Biology

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4
PROFESSIONAL: 4
OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two areas in mammary gland biology are of interest to this laboratory. The mechanisms employed to activate transcription of milk protein genes during pregnancy, and the genetic switches that turn the mammary anlage into a functional organ. The whey acidic protein (WAP) gene serves as a paradigm to understand gene regulation in the mammary gland. Genetic switches and mechanisms have been identified which control the regulation of the WAP gene. Activation of WAP genes during pregnancy is mediated through transcription factors from the Ets family which bind to a specific sequence in the promoter. This illustrated that Ets signalling pathways can function as stage specific transcriptional activators in the mammary gland during pregnancy.

Experiments were performed to understand genetic pathways which mediate the growth and differentiation of mammary tissue during puberty and pregnancy. It was determined at the molecular level that the mammary gland goes through several differentiation phases during development. Mammary epithelial cells undergo secretory differentiation in virgins during the estrus cycle, but require pregnancy for the establishment of terminal differentiation. These mechanistic studies provided the explanation for earlier findings by us and others that virgin transgenic mice carrying oncogenes can develop tumors, just like in human. In these cases oncogenes are activated during the estrus cycle in virgins and transform cells prior to pregnancy. Studies on mice with a non-functional CSF-1 gene revealed that CSF-1 is required for functional differentiation of mammary tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 18008-29 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cell Regulation by Hormones, Growth Factors, Autoanitbodies, and Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. D. Kohn	Medical Director, USPHS & Chief, Sec. on Cell Regulation	LBM, NIDDK
Others:	M. Ohmori	Visiting Fellow	LBM, NIDDK
	S.-I. Taniguchi	Visiting Fellow	LBM, NIDDK
	M. Ohta	Visiting Fellow	LBM, NIDDK
	M. Shong	Visiting Fellow(6 months)	LBM, NIDDK

COOPERATING UNITS (if any)
NCI (D. Singer, S. Kimura); NICHD (G. Cutler); U. Texas Galveston (B. Prabhakar); U. MD Med School (W. A. Valente); John Hopkins Med. School, MD (M. Saji, M. Zeiger, M. Levine, N. Rose); U. Naples Med School, Italy (S. Aloj, E. Consiglio); U. Udine Med School, Italy (F. S. Ambesi-Impiombato)-Continued on Page 2

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section of Cell Regulation

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 9.25	PROFESSIONAL: 8.5	OTHER: .75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This lab has further defined structure-function relationships of the thyrotropin receptor (TSHR) with respect to the following: TSH binding; signal transduction; cell cycle-coordinated, transcriptional regulation of thyroid growth and differentiation; relationships to gonadotropin receptor structure, function, and gonadal hyperfunction in precocious puberty; the action of autoantibodies causing autoimmune thyroid disease; the basis for the development of TSHR autoantibodies; and the link of thyroid autoimmunity to other autoimmune diseases. Epitopes for thyroid stimulating, thyroid blocking, and TSH binding inhibiting autoantibodies have been defined and related to the immunodominant peptide of the receptor. Factors and elements on the minimal TSHR promoter which are important for regulation of growth and function have been identified, characterized, and cloned, including unusual single-strand binding trans factors. These factors have been shown to coregulate TSHR and major histocompatibility (MHC) gene expression, class I and class II, as a function of the cell cycle. Autoimmune thyroid disease is postulated to result from loss of the coordinated negative regulation of TSHR, MHC class I, and MHC class II; too much negative regulation is linked to tumor development. A drug, methimizole (MMI), useful for Graves' disease, has been shown to be effective in experimental lupus and blepharitis; its sites of action on MHC class I and class II defined; and methods to develop better immunosuppressive drugs in this class, therefore, established. A new assay system using TSHR chimeric receptors has been developed which appears to prospectively identify Graves' patients sensitive to remission by MMI therapy alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 18009-16 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Endocytosis, Secretion and Compartmentalization in Mutant CHO Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.R. Robbins	Research Geneticist	LBM, NIDDK
Others:	C.W. Hall	Research Chemist	LBM, NIDDK
	R. D. Ward	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)
Hematology-Oncology Section, Department of Medicine, Baylor College of Medicine (Dr. Daniel Epner)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued our analysis of the mechanism(s) by which a single amino acid substitution in 15-25% of CHO cells' glyceraldehyde 3-phosphate dehydrogenase (GAPDH) polypeptides results in altered morphology, mobility and distribution of late endocytic organelles. Our results indicate that both mutant and wild type GAPDH are bound to microtubules in semi-intact cell preparations. In cells expressing mutant GAPDH, organization of the cytoskeleton is altered: microtubule bundles are present, thin filaments, as yet unidentified, extend from these bundles and appear to attach to endosomal compartments clustered at the cell poles.

The mutant GAPDH we are studying contains a substitution at an amino acid that has been conserved throughout evolution. We have examined the effects of this mutation on enzyme function, comparing purifed homotetramers of mutant and wild type GAPDH. The mutant enzyme manifests one-half the normal activity; no significant differences were observed in either substrate or cofactor concentration dependence. A hallmark of the mutant enzyme is that its binding to microtubules is resistant to dissociation by ATP; in contrast, its enzymatic activity shows a >50-fold increase in sensitivity to ATP.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-920

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 18010-08 LBM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Intracellular Traffic in HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J.A. Hanover	Research Chemist	LBM, NIDDK
Others:	D.W. Frank	IRTA	LBM, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Cell Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01-DK 60001-01 LCBB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 18017-02 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Recombinase-mediated control of gene expression and genome structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian Sauer	Expert	LBM, NIDDK
Others:	S. Gagneten	IRTA	LBM, NIDDK
	B. Bethke	IRTA	LBM, NIDDK
	J. Sztein-departed 6/30/95	Visiting Associate	LBM, NIDDK
	T. Larson-arrived 6/26/95	Biologist	LBM, NIDDK

COOPERATING UNITS (if any)
LBM, NIDDK (Gilbert Ashwell); NICHD (Heiner Westpah); NIMH (Ute Hochgeschwender),NIDR (Jerry Keith); U. Southern California (Michael Ignelzi); U. of Cologne (Klaus Rajewsky); DuPont Merck (Ron Hoess)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4
PROFESSIONAL: 4
OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Generation of transgenic animal models and production of useful genetically engineered mammalian cell lines is highly dependent on endogenous DNA recombination mechanisms (either homologous recombination or illegitimate recombination) for stable incorporation of exogenous DNA into the genome. An alternative and complementary recombination methodology based on the prokaryotic site-specific recombinase Cre from phage P1. Expression of Cre recombinase in mammalian cells catalyzes DNA recombination between specific 34 bp loxP sites that were previously engineered into the genome by standard gene transfer methodologies. Because these sites do not exist naturally in the mouse genome, recombination occurs specifically at the sites placed into the genome. By simply choosing how and where such sites are placed on the DNA, Cre recombinase can specifically and precisely delete a preselected DNA segment from the genome. Moreover, because Cre also catalyzes intermolecular recombination, exogenous DNA containing a loxP site can be precisely targeted for integration at a loxP site previously placed into the genome, thus allowing the facile production of isogenic genetically engineered cell lines.

This methodology has now been used to remove unwanted DNA (such as selectable markers) from the genomes of yeast and transgenic mice. Moreover, this recombination system was exploited to design a binary transgenic mouse scheme that allows the controlled expression of potentially toxic genes or that can be used to generate a somatic knockout mutation. Combining this binary recombination system with one of the numerous inducible gene expression systems has the potential of allowing the generation of a somatic knockout in a transgenic animal by the simple administration of a chemical compound.

U.S. GOVERNMENT PRINTING OFFICE [illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 18018-01 LBM

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Fibroblast Growth Factor Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chuxia Deng	Tenure Track Investigator	LBM, NIDDK
Others:	ZiYao Liu	IRTA	LBM, NIDDK
	Xiaoling Xu	Visiting Associate	LBM, NIDDK

COOPERATING UNITS (if any)
Baylor College of Medicine (Stephen J. Elledge, J. Wade Harper), Harvard Medical School (Philip Leder), NCHGR (Tony Wynshaw-Boris)

LAB/BRANCH Laboratory of Biochemistry and Metabolism

SECTION Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary interest of this laboratory is mammalian development and oncogenesis using both knockouts and transgenic animal model systems.

Studies have being carried out on functions of fibroblast growth factor receptors (FGFRs) in mammalian development. FGFRs constitute a family of four membrane-spanning tyrosine kinases (FGFR1-4) which serve as high affinity receptors for at least nine growth factors (FGF1-9). FGFs/FGFRs signals may have many important biological functions, including developmental induction and differentiation, wound healing, tumor angiogenesis, cell growth and migration, as well as neuronal differentiation and survival. Moreover, it has been recently demonstrated that alterations in FGFRs are responsible for at least seven human inherited diseases. All of these diseases are caused by single amino acid mutations and exhibit extensive craniofacial or axial and appendicular bone abnormalities. To study functions of FGFs/FGFRs signals in development and to gain insight into mechanism(s) underlying these inherited diseases, mice have been created that carry targeted disruption of each individual receptor through homologous recombination in ES cells. Analyses of these mutant mice revealed each of the FGF receptor to have distinct functions during murine development: FGFR-1 plays a role in axial organization during gastrulation; FGFR-2 plays a role at postgastrulation stages; FGFR-3 is a negative regulator in bone growth and development; and both FGFR-3 and FGFR-4 work together to monitor postnatal growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 21019-13 LCBG

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Hormone and Transmitter Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Harvey B. Pollard Medical Officer LCBG:NIDDK

Others: I. Atwater, Ph.D., Res. Bio; M. Levine, M.D., Sen. Inv., Med. Off.; M. Srivastava, Ph.D., SSF; G. Lee, Ph.D., Res. Chem., E. Rojas, Ph.D., VS; C. McCutchen, Ph.D., Res. Phys; G. Kuijpers, Ph.D., VA, G. Goping, EM Tech.; F. Vargas, DDS, FDA; K. Dhariwal, Ph.D., SSF; R. Welch, Ph.D., IRTA; Y-H Wang, M.D., IRTA; H. Caohuy, B.S., Biolo., C. Guay-Broder, B.S., Biolo.; N. Aripse, Ph.D., VA; Y. Raviv, Ph.D., SV; L. Vergara, M.D., VS; O. Eidelman, Ph.D., SV; R. Vinet, Ph.D., SV; E. Cohen, Ph.D., SV; S. Bar-Noy, Ph.D., SV; M. Glasman, Ph.D., VA; Merezhinskaya, Ph.D., VA; J. Park, Ph.D, IRTA, S. Rumsey, Ph.D., IRTA, Nachson, S., Ph.D., SV

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Cell Biology and Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Building 8, Room 403, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human and mouse synexin genes were sequenced, and assigned to homologous chromosomes. The human gene was located at position 10q21.1-21.2, while the mouse gene was located on chromosome homologous 14. The synexin genes were found to be highly homologous in terms of splice junction location, but quite distinct from the organization of other members of the annexin family. The half life of synexin was found to be ca. 40 h, and inhibitors of protein synthesis blocked secretion from bovine chromaffin cells in an appreciably shorter time. Thus, in addition to synexin other proteins are required for the secretory process. Human cytochrome b_{561}, a major transmembrane protein of chromaffin granules, was found to have 5 transmembrane domains rather than the six previously hypothesized. Membrane potential was found to regulate cytoplasmic calcium oscillations in pituitary gonadoptrophs. M_3-muscarinic receptors were found to potentiate glucose-induced insulin release from rat islets. The diabetogenic agent alloxan was found to activate K(ATP) channels in mouse pancreatic beta cells. A novel cerebrospinal/vascular shunt was developed to implant xenobiotic islets in diabetic animals. Human pancreatic islets were analyzed by electrophysiologic methods for the first time. Evidence was obtained that I_{CRAC} currents control electrical activity in islets through effects on cholinergic receptors. A geometric sequence (the three-halves rule) was developed which accurately describes allowed multiple conductance states of ion channels. A theoretical model of the ion channel structure of the amyloid beta protein was constructed. A xanthine drug (CPX) and an analog compound (DAX) were found to activate chloride efflux from cystic fibrosis cells. An IND was filed with the FDA and clinical testing is planned. Uptake of ascorbate and dehydroascorbate were found to be taken up by fibroblasts by separate mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 22001-04 LCBG

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vitamin C: Biochemistry, Molecular Biology, and Human Requirements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Mark Levine, Senior Investigator, LCBG:NIDDK
Others: Kuldeep Dhariwal, Senior Staff Fellow, LCBG:NIDDK
Jae Park, IRTA, LCBG:NIDDK
Richard Welch, IRTA, LCBG:NIDDK
Yaohui Wang, Visiting Fellow, LCBG:NIDDK
Yukio Yamada, Special Volunteer, LCBG:NIDDK

COOPERATING UNITS *(if any)*
L. Cantilena (USUHS, Bethesda), C. Cantilena (Transfusion Medicine, CC, NIH), L. Helman (PB, NCI, NIH), K. Kirk (LBC, NIDDK, NIH), A. Crossman (LBC, NIDDK, NIH), S. Chanock (PB, NCI, NIH)

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The recommended dietary allowance for vitamin C (ascorbate) is based on preventing the deficiency disease scurvy. We proposed that a new recommended dietary allowance for vitamin C requirements can be determined using the concept of in situ kinetics.

In situ kinetics has biochemical and clinical components. The goal of the biochemical component is to determine vitamin C molecular function in relation to its concentration. For these studies vitamin C function is investigated in human fibroblasts, lymphocytes, and neutrophils. To determine how intracellular concentration is regulated, vitamin transport has been characterized. Ascorbate transport is concentration dependent, saturable, mediated by a sodium-dependent carrier, and inhibited by newly synthesized vitamin C analogs. Oxidized ascorbate dehydroascorbic acid, is transported separately by GLUT I and immediately reduced intracellularly to ascorbate. Several proteins have been isolated which mediate dehydroascorbic acid reduction to ascorbate in neutrophils. One protein was responsible for the majority of reducing activity and was isolated, sequenced, identified as thioltransferese (glutaredoxin), cloned, and modified by site-directed mutagenesis. Cloning of genomic glutaredoxin and characterization of the promotor region are underway. These studies indicate that vitamin C function in neutrophils may be to protect neutrophils from their own oxidants. Overall findings for fibroblasts, neutrophils, and lymphocytes suggest that kinetics for vitamin C function in situ can be determined in relationship to vitamin concentration in situ.

The clinical component of in situ kinetics is to determine how vitamin concentrations are achieved in normal humans as a function of dose and whether concentrations humans achieve are those required for molecular functions of the vitamin. A clinical trial completed in healthy men invested the relationship between vitamin C dose and its concentration in plasma and tissues, bioavailability, urinary excretion, and potential adverse effects. A clinical trial in women is underway.

Based on biochemical and clinical in situ kinetics, we are recommending a new recommended dietary allowance for vitamin C in healthy people.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,330-17 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Permeability Barriers of Gram-Negatives and Mycobacteria to Antibiotics and Chemotherapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William G. Coleman, Jr., Ph.D.	Research Microbiologist	LBP NIDDK
Others:	Lishi Chen, Ph.D.	Sr. Staff Fellow	LBP NIDDK
	Li Ding, Ph.D.	IRTA Fellow	LBP NIDDK
	Keith Crawford, Ph.D.	IRTA Fellow	LBP NIDDK
	Dorothy Paul	Pre-IRTA	LBP NIDDK
	Thomas Klein	Special Volunteer	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.7	3.7	2.0

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues [✓ (c) Neither]

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The *rfaD* gene and its gene product, ADP-L-glycero-D-mannoheptose 6-epimerase have been defined and reported by my laboratory. Procedures for crystallization of the epimerase have successfully yielded three crystal forms of the enzyme adequate for preliminary X-ray diffraction analysis. One of the crystal forms (forms III) was obtained by the hanging drop vapor diffusion method in the presence of ADP-glucose (a catalytic inhibitor of the epimerase) and the precipitant, polyethylene glycol 400. The form III crystal appears to belong to the trigonal space group $P3_1$, with unit cell dimensions of a=b=114 Å, c=179 Å. This crystal diffracts x-ray to at least 2.8 Å. High-resolution structure determination is ongoing. We have demonstrated structural and functional homologies of the *rfaD* gene product of *Brucella abortus*, *Pseudomonas aeruginosa*, and *Neissera gonorrhoeae* to its *E. coli* K-12 counterpart.

Physicochemical studies of the *rfaC* product, heptosyl transferase I, are ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,580-32 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Transposons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony V. Furano, M.D.	Medical Officer (Research) and Chief, Section on Genomic Structure and Function	LBP NIDDK
Others:	Eric Cabot, Ph.D.	IRTA Fellow	LBP NIDDK
	Olivier Verneau, Ph.D.	Special Volunteer	LBP NIDDK
	Brian Good	IRTA Exception	LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. Francois Catzeflis, Institute of Evolutionary Science, Montpellier, France; Dr. Karen Usdin, NIDDK; Dr. Leslie Derr, NIAID

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1

CHECK APPROPRIATE BOX(ES

(a) Human subjects   (b) Human tissues   [✓ (c) Neither]

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

L1 retrotransposable elements account for at least 30% of all mammalian genomes. L1 transposition is active in present day mammals and causes up to 0.2% of the genetic defects in humans. Full length mammalian L1 elements are 6-7 kb and all share the same organization: a 5' UTR which includes a regulatory sequence; ORF I, which encodes a protein of unknown function; ORF II which encodes a reverse transcriptase; and a 3' UTR that contains a G-rich polypurine:polypyrimidine tract and terminates in an A-rich sequence. Most of what is known about the mechanism of L1 regulation, replication, and transposition has been derived from evolutionary studies carried out by us and others. These studies have generated two kinds of information: First, comparisons between different L1 families or L1-like elements has identified and assigned possible functional significance to the various L1 structural components. Second, it is now clear that L1 evolution is a paradigm for a novel evolutionary process that is taking place within the "ecosystem" of the mammalian genome and that L1 evolution is quite dynamic, with novel L1 variants continually emerging over relatively short periods of time. As a consequence, L1 evolution has generated a rather complex family structure and we have exploited this feature of L1 DNA evolution to examine the phylogenetic history of their mammalian hosts. Over the past year we have extended our knowledge on the properties of the L1 regulatory sequence, on the evolutionary dynamics of L1 elements and more fully developed the use of L1 DNA as a phylogenetic tool. We previously showed that the regulatory region of the rat L1 element strongly stimulates the activity of a gene fused to it which was the first evidence that L1 DNA is not just some non-functional "junk" DNA. We now know that the L1 regulatory region is not a typical transcriptional activator sequence but may stimulate gene activity by acting as an activator of protein translation. We have identified a hypervariable region in that part of the ORF I sequence that encodes glutamine motifs, suggesting that this motif is important for the function of ORF I. We also found that closely related L1 subfamilies may compete with each other and that L1 families may retain some transpositional activity for millions of years. And, finally, we have used L1 DNA as a phylogenetic tool to determine the phylogeny of *Rattus* sensu strictu a group that so far has been refractory to standard taxonomic techniques.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 23,900-04 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Function of Microtubules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jan Wolff, M.D., Ph.D.	Medical Officer and Chief, Section on Endocrine Biochemistry	LBP NIDDK
Others:	Dan Sackett, Ph.D.	Expert	LBP NIDDK
	Shobha Uppuluri, Ph.D.	Visiting Associate	LBP NIDDK
	Sadananda Rai, Ph.D.	Visiting Fellow	LBP NIDDK
	Leslie Knipling	Biologist	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Endocrine Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES
◇ (a) Human subjects ◇ (b) Human tissues X (c) Neither
◇ (a1) Minors
◇ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The properties of ligand binding sites on the tubulin dimer have been studied in detail. Colchicine binds to two peptides on β-tubulin near the α/β interface of the constituent monomers. α-binding domains have also been identified but some are nonspecific. Colchicine binding leads to unfolding of an amphipathic helix of β-tubulin exposing new proteolytic binding sites at Phe-389 and Lys 392. Members of the vinca alkaloid group, which bind at a different site, block and prevent the local unfolding induced by colchicine, and the associated GTPase activity of tubulin. The two drugs can also be differentiated by their different effects on intrinsic tryptophane fluorescence. A fluorescent vinblastine analogue has been synthesized and is being used to localize the binding site.

The high sensitivity of tubulin polymerization to urea (I_{50} ~250 mM) has now been compared with the effects of guanidine hydrochloride. This denaturant has a polymerization promoting activity between 10-120 mM leading to a different polymer. Larger concentrations unfold the protein as expected. The guanidine effect is mimicked by a number of other cations and we believe these agents shield the repulsive amionic charges which destablize the polymer and thus promote polymerization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,140-29 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of the Tryptophan Synthase Multienzyme Complex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edith Wilson Miles, Ph.D.	Research Chemist and Chief, Section on Enzyme Structure and Function	LBP NIDDK
Others:	Syed A. Ahmed, Ph.D.	Senior Staff Fellow	LBP NIDDK
	Utpal Banik, Ph.D.	Visiting Fellow	LBP NIDDK
	Li-hong Yang, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)* Drs. D.R. Davies, S. Rhee & K.D. Parris, LMB, NIDDK; C.C. Hyde, LSBR, NIAMS; P. McPhie, D.L. Sackett, A. Minton & D. Millar, LBP, NIDDK; A. Ginsburg, D.P. Remeta, D.-M. Zhu & P.B. Chock, LB, NHLBI; J.R. Knutson & L. Davenport, IR, NHLBI; E. Woehl & M.F. Dunn, Univ. Calif., Riverside; A. Mozzarelli & G.L. Rossi, Univ. of Parma, Italy; K.S. Anderson & A.Y. Kim, Yale Univ., New Haven, CT; I. Schlicting, Max Planck Institute, Heidelberg, Germany

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Enzyme Structure and Function

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	

CHECK APPROPRIATE BOXES

☐ (a) Human subjects ☐ (b) Human tissues ✓ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)* The trytophan synthase multienzyme $\alpha_2\beta_2$ complex provides a useful model system for us to investigate how enzymes interact, communicate and channel reaction intermediates. We are using the crystal structure of the tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* as the basis for designing mutations to further investigate the structure and function of tryptophan synthase. During the past year we have made several major findings. (1) We find that Cs^+ stimulates the β subunit activity of the wild type $\alpha_2\beta_2$ complex and partially restores the activity of several low activity mutant forms of tryptophan synthase. These results suggest that Cs^+ stabilizes the active conformation of the wild type and mutant β subunits. Crystallographic studies have identified a cation binding site in the β subunit. (2) To probe tunnel function, we have designed mutations (β-C170F and β-C170W) to block or restrict the unique tunnel which connects the active sites of the α and β subunits in the $\alpha_2\beta_2$ complex. Kinetic analyses of the mutant $\alpha_2\beta_2$ complexes show that the tunnel is restricted and provide direct evidence for the substrate channeling mechanism. (3) Studies using Nile Red as a sensitive fluorescent probe of the hydrophobic tunnel reveal that allosteric ligands (α subunit) or active site ligands (β subunit) induce conformational changes in the tunnel that may control indole channeling. (4) To better understand the mechanism of subunit assembly, we have probed the interaction of the α subunit with wild type and mutant (β-K87T) subunits by activity measurements and by gel filtration and subunit interchange experiments. The results indicate that formation of interdomain bridges by pyridoxal phosphate-aldimine intermediates at the β site stabilizes subunit association and stabilizes pyridoxal phosphate binding. (5) Our analysis of the thermally-induced unfolding of the separate α and β subunits and $\alpha_2\beta_2$ complex by UV-visible and circular dichroism spectroscopy, activity measurements and differential scanning calorimetry allows us to correlate thermodynamic and structural information.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,150-24 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Noncovalent Intermolecular Interactions in Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen P. Minton, Ph.D.	Research Chemist and Chief, Section on Physical Biochemistry	LBP NIDDK
Others:	Jianqing Wu, Ph.D.	IRTA	LBP NIDDK
	Peter Schuck, Ph.D.	Visiting Fellow	LBP NIDDK
	David Millar, Ph.D.	Courtesy Associate	LBP NIDDK
	Saleh Darawshe, Ph.D.	Courtesy Associate	LBP NIDDK

COOPERATING UNITS *(if any)* R. C. Chatelier, CSIRO, Clayton, VIC, Australia; S. A. Ahmed, LBP, NIDDK

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	

CHECK APPROPRIATE BOXES

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The time-dependent behavior of macromolecules binding to receptors immobilized within a polymer matrix was explored via computer simulation. The literature on determination of chemical kinetics using biosensor instrumentation has been reviewed in light of the results obtained in the simulations.

Development of an automated technique for the quantitative determination of unprecedentedly small amounts of unlabeled proteins in aqueous solution, and its application to the quantitation of protein gradients in a centrifuge tube continues.

The equilibrium dissociation of the holoenzyme tryptophan synthase into monomeric alpha and dimeric beta subunits was studied via sedimentation equilibrium.

A mechanistic model for the dependence of the osmotic pressure of serum albumin upon concentration and pH has been developed.

A scaled particle theory for the thermodynamic behavior of a two dimensional fluid of hard convex particles has been developed.

Computer programs for the analysis of sedimentation equilibrium of solutions of a single macrosolute have been extended to treat additional models.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,590-24 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Interactions of Biologically Important Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Harry A. Saroff, Ph.D. Research Chemist (Intermittent) LBP NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues [✓ (c) Neither]
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems, particularly those involving protein-DNA interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,709-14 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polyamine Biosynthesis and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Celia White Tabor, M.D.	Medical Officer (Research)	LBP NIDDK
Others:	Herbert Tabor, M.D.	Supervisory Medical Officer (Research) Chief, Section on Pharmacology, LBP; and Chief, Laboratory of Biochemical Pharmacology	LBP NIDDK
	David Balasundaram, Ph.D.	Visiting Associate	LBP NIDDK
	Nobuko Hamasaki, Ph.D.	IRTA	LBP NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.7	

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues √(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Putrescine, spermidine, and spermine are major polybasic compounds that are found in all living cells. Work from this and other laboratories have shown that these amines are important for many systems related to growth and differentiation. Our studies are directed towards learning more about how these polyamines are synthesized, how their biosynthesis and degradation are regulated, their physiological functions, and the mechanisms of their actions *in vivo*. Our present studies have concentrated on the biochemistry, regulation, and genetics of these amines in *Saccharomyces cerevisiae*. For this purpose we have used null mutants of this organism that we have constructed in the various biosynthetic pathways. These mutants are not able to make the polyamines and, therefore, are very useful tools for these physiological studies. Work with these mutants have shown a number of interesting and important *in vivo* functions for the polyamines. In particular, our work has indicated that polyamines are required for growth, for sporulation, for maintenance of the killer dsRNA virus, for protection against oxidative damage, for protection against elevated temperatures, for fidelity of protein biosynthesis, and for the stability of ribosomes and of the ribosome-aminoacytRNA-mRNA complex. Our work during the past year has shown that the different polyamines have different effects and that the requirement for spermidine could not be replaced by putrescine or spermine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,940-22 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Yeast RNA Virology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D.	Medical Officer, USPHS and Chief, Section on Genetics of Simple Eukaryotes	LBP NIDDK
Others:	Jonathan D. Dinman, Ph.D.	Staff Fellow	LBP NIDDK
	Rosaura P. C. Valle, Ph.D.	Visiting Fellow	LBP NIDDK
	Juan Carlos Ribas, Ph.D.	Visiting Fellow	LBP NIDDK
	Herman Edskes, Ph.D.	Visiting Fellow	LBP NIDDK
	Kathleen Carroll, Ph.D.	IRTA Fellow	LBP NIDDK
	Daniel Masison, Ph.D.	IRTA Fellow	LBP NIDDK

COOPERATING UNITS *(if any)* A. Steven, LSB, NIAMS; J. Johnson, Purdue Univ.

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	5.6	

CHECK APPROPRIATE BOX(ES

◇ (a) Human subjects ◇ (b) Human tissues √ (c) Neither

◇ (a1) Minors

◇ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have described four yeast RNA replicons, namely the L-A and L-BC dsRNA viruses, and the ssRNA replicons, 20S RNA and 23S RNA. The propagation of all four of these replicons is partially blocked by the *SKI2*, *SKI3*, *SKI4*, *SKI6*, *SKI7* and *SKI8* genes, which we have shown act by blocking the translation of poly(A)$^-$ mRNAs, such as the viral messages. Two close human homologues of *SKI2* have been described, one of which has been shown to be a nucleolar protein. We have found that the concentration of free 60S ribosomal subunits is critical for L-A virus propagation, probably because of a role of the 3' poly(A) structure of the mRNA in attracting and activating 60S subunits for translation.

We have previously characterized the *SKI2*, *3*, and *8* genes, and we have now cloned the *SKI6* and *SKI7* genes. Their sequencing and characterization will further define this important system.

We find that the L-A virus has an icosahedral structure with T=1 and an assymetric unit comprised of a dimer of Gag, the major coat protein. Preliminary studies by others indicate that the core of reovirus and rotavirus have essentially the same structure.

We are studying the transcriptase activity of the L-A virus, using our template dependent system. We find that this enzyme recognizes a signal within the first 18 bp of the L-A dsRNA structure. We have also detected the RNA-dependent RNA polymerases of 20S RNA and of the L-BC virus and are studying their specificities.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,941-04 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membranes Cytoskeleton and Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jan Wolff, M.D., Ph.D.	Medical Officer and Chief, Section on Endocrine Biochemistry	LBP NIDDK
Others:	Leslie Knipling	Biologist	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Endocrine Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues x (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive this year but being resumed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK24,942-03 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter McPhie, Ph.D. Research Chemist LBP NIDDK

COOPERATING UNITS *(if any)*
Dr. E. W. Miles, LBP; Dr. W. Jakoby, LBM, NIDDK; Drs. S-y. Cheng, S. Adhya, I. Pastan, LMB, NCI; Dr. D. Mahadevan, LCMB, NCI

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Enzyme Structure and Function

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES

(a) Human subjects (b) Human tissues [✓ (c) Neither]
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory is engaged in studies on protein structure and the mechanism of protein folding. The main subject of research is swine pepsinogen, a monomeric protein of molecular weight = 39,630, which is stable at pH's between 6 and 8.5. Below pH 6 pepsinogen activates itself by proteolytic loss of it's first 44 amino acids, to produce an enzymatically active protein, pepsin. Pepsin is stable only at pH's below 6. Pepsin and pepsinogen are unfolded by exposure to high pH, temperature or concentrations of denaturants, such as urea. However, unfolded pepsinogen can refold to its normal structure, when returned to native conditions, whereas pepsin cannot. I am interested in the mechanism of this refolding reaction and how the difference in sequence influences the refolding of the two proteins. We have used rapid kinetic techniques, (stopped-flow and T-jump), to detect partly folded intermediate forms in the folding reaction; their structures have been partially determined and the nature of the chemical reactions which separate them from the native and unfolded forms investigated. Techniques such as ultra-violet absorbance, circular dichroic and fluorescence spectroscopies, together with chemical modification and peptide chemistry, to characterize the structures of native and unfolded forms of these and other proteins, including tryptophan synthase, human nuclear thyroid hormone receptor and sulphotransferase enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 24,943-02 LBP

PERIOD COVERED
October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prions of Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D.	Medical Officer, USPHS and Chief, Section on Genetics of Simple Eukaryotes	LBP NIDDK
Others:	Daniel C. Masison, Ph.D.	IRTA	LBP NIDDK
	Herman Edskes, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	

CHECK APPROPRIATE BOX(ES

◊ (a) Human subjects ◊ (b) Human tissues ✓ (c) Neither
◊ (a1) Minors
◊ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A prion is an infectious protein, an altered form of a normal protein which has lost its normal function but has acquired the ability to convert the normal form of the protein into this same altered (prion) form. This concept arose in studies of scrapie, a CNS disease of sheep, and the same disease of man (kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Streussler-Scheinker (GSS) disease). We have proposed, based on genetic evidence, that the yeast non-Mendelian elements, [URE3] and [PSI] are prions, infectious altered forms of the cellular Ure2p and Sup35p, respectively [Wickner, R. B.: *Science* **264**, 566-569 (1994)]. Ure2p is a negative regulator of transcription of genes encoding enzymes for the catabolism of poor nitrogen sources. Sup35p is involved in the termination of translation.

We find that overexpression of the entire Ure2p produces a 100-fold increase in the frequency with which [URE3] arises. Overexpression of just the N-terminal 65 residues of Ure2p is sufficient to induce [URE3] and the frequency is 8000-fold above the backround level. Overexpression of the C-terminal part, lacking just the N-terminal 65 residues is sufficient to carry out the normal nitrogen catabolite repression activity of Ure2p, and this C-terminal domain cannot induce the prion change and cannot undergo the prion change itself.

The earliest biochemical finding implicating the mammalian PrP in the scrapie phenomenon was that this protein was protease K-resistant in the brains of sheep with scrapie, but sensitive in normal sheep. We find that the Ure2p in extracts of [URE3] strains is much more protease K-resistant in comparison with wild-type [ure-o] strains. The fragments that are resistant to proteinase K digestion include the N-terminal prion-inducing domain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,944-01 LBP

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Unusual Nucleic Acid Structures in Normal and Pathological Biological Processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Usdin, Ph.D.	Visiting Associate	LBP NIDDK
Others:	Neale Weitzmann, Ph.D.	Visiting Fellow	LBP NIDDK
	Edward Grabczyk, Ph.D.	IRTA Fellow	LBP NIDDK
	Renée Howell, Ph.D.	IRTA Fellow	LBP NIDDK
	Kerry Woodford	Pre-IRTA	LBP NIDDK

COOPERATING UNITS *(if any)* Dr. R. Nussbaum, National Center for Human Genome Research; Dr. L. Derr, NIAID; Dr. A. V. Furano, NIDDK; Dr. F. Catzeflis, Institut des Sciences de l'Evolution, Université Montpellier II, France

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES

(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

√ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)* We have been studying the effect of DNA and RNA secondary structure on gene expression, DNA replication and mutation. In particular we have been examining the role of unusual nucleic acid structures in the etiology of fragile X syndrome as well as a number of other genetic defects. Fragile X syndrome is the most common cause of inherited mental retardation, occurring at a frequency of about 1 in 1500 males and 1 in 2500 females. Most mutations associated with this syndrome coincide with gaps of poorly staining chromatin on the X chromosome seen when cells containing this mutation are starved for the precursors of DNA synthesis. These so-called fragile sites are associated with a sudden, large increase in the number of repeats of the triplet CGG found in the 5' untranslated region of a gene called FMR-1. This so-called triplet expansion results in increased methylation of the FMR-1 promoter and the absence of detectable mRNA. Six other triplet expansion diseases are currently known, that are due either to expansion of CGG-repeats, or to the expansion of a repeat with the sequence CAG.

We have shown that CGG-repeats can form a number of unusual nucleic acid structures, including an unusual hairpin in the absence of K^+, and an intrastrand tetraplex containing both guanine tetrads and C•C basepairs in the presence of this cation. The tetraplex forms a strong block to DNA synthesis that might provide a biological rationale for triplet expansion by promoting repeated strand slippage during replication. We have shown that similar structures are also found associated with other hypervariable sequences where it is possible that they affect DNA stability in much the same way.

Other work in progress in this group includes a study of a conserved RNA structure found in non-LTR retrotransposons in mammals, a study of an unusual DNA structure we call a cinched tetraplex in the promoter of the chicken β-globin gene, and a study of the effect of triplex formation on transcription *in vitro* and *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25011-20 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of Protein Folding

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hiroshi Taniuchi	Chief, Protein Chemistry and Conformation Section	LCB/NIDDK
Others:	Ying Shi	Visiting Fellow	LCB/NIDDK
	Alice Hawley	Chemist	LCB/NIDDK
	Greg Charles	Biological Aid	LCB/NIDDK
	Xio-Ying Ge	Special Volunteer	LCB/NIDDK

COOPERATING UNITS (if any)
Structural Biophysics Section, Laboratory of Biophysical Chemistry, NHLBI, Biotechnology Unit, Laboratory of Cellular and Developmental Biology, NIDDK

LAB/BRANCH Laboratory of Chemical Biology

SECTION Protein Chemistry and Conformation Section

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 3.2	PROFESSIONAL: 2.6	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous years we have proposed a model of four core domains of cytochrome c (cyt.c) fragment complexes whose interactions drive folding. A core domain is a structural region containing a hydrophobic core and the surrounding shell, it folds and unfolds as a unit. To gain insight into this hypothetical core domain-domain interaction we have carried out site directed mutagenesis of yeast iso-2 cyt. c: single substitutions L9I, I20V, M64L, L85I, and M98L, double L9I/M64L, I20V/M98L, and M64L/M98L, triple L9I/I20V/M98L, L9I/M64L/M98L, and I20V/M64L/M98L, quadruple L9I/I20V/M64L/M98L, and quintuple L9I/I20V/M64L/L85I/M98L. These residues are located in the core or partly in the core. Shell residue substitutions T99K and E22K were also performed. All these substitutions are those which occur going from yeast iso-2 to horse cyt.c. Despite these 'native' substitutions, single mutant I20V and quadruple mutant L9I/I20V/M64L/M98L iso-2 cyts. c are found to be non-functional (yeast cells containing phagemids bearing the mutant gene fail to grow on non-fermentable carbon source, glycerol), while all the other mutants iso-2 cyts.c are functional. Thermal transition of the 695 nm absorption band of the wild type and mutant cyts. c, indicative of the Met 80S-heme Fe bond, show that there are interactions between Ile 20 and Met 98 and between Met 64 and Met 98. The pattern of this transition and the data of the 628 nm absorbance band of the mutant cyts. c also suggest that residues 9 and 64 may influence each others substitution effect. Examination of the 655 nm absorption band of the wild type and mutant cyts. c suggests that an increase in the intensity of this band partially correlates with the loss of function. All these observations can be explained by assuming that there may be some long-range interaction-network associated with the packed or ordered core residues of yeast iso-2-cyt. c that modulates the stability of the conformation and the function. It is also assumed that these interactions are sensitive to substitution of the residues involved. Consistently, the collaborative two-dimensional NMR studies of the wild type and M64L, M98L and M64L/M98L mutant iso-2 cyts. c suggest that perturbation of the core residue packing by these substitutions, particularly by the double substitution significantly increases conformational mobility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25016-21 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cis Acting Factors Controlling Globin Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jin Li	IRTA Fellow	LCB/NIDDK
Others:	Pranvera Ikonomi	Visiting Fellow	LCB/NIDDK
	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
	Alan N. Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)
Hematology Division, University of Athens School of Medicine, Athens, Greece (Dr. Panagoula Kollia)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The first of two developmental switches in human hemoglobin expression involves the down-regulation or silencing of the embryonic globin genes. We have previously identified a developmental silencer located around 250 bp 5' of the cap site of the epsilon globin gene which contains binding sites for transcription factors, GATA-1 and YY1. This negative regulatory element silences epsilon promoter activity in primary human adult erythroid cells and in adult transgenic mice, and promoter activity is restored by mutations in the GATA-1 and YY1 binding sites. These studies show that GATA-1 acting with other cell specific proteins can participate in both negative and positive globin regulation as co-transfection with a GATA-1 expression vector increases epsilon-globin transcription in K562 cells which express embryonic globins. Further examination of the endogenous epsilon-globin gene in adult erythroid progenitors indicates that epsilon-globin remains active, but at low levels, and produces aberrant transcripts, possibly by transcriptional pausing or inefficient splicing mechanisms. These studies suggest a role for events after transcription initiation in hemoglobin switching and may be possible targets for therapeutic intervention in hemoglobinopathies. Recent examination of other DNA regulatory regions within the 5' flanking region of the epsilon globin gene suggest negative elements located 3 kb and 1.7 kb 5' of the cap site, the latter appears to be erythroid specific. DNase I footprinting reveals several potential protein binding sites in the 3 kb 5' negative element. These results indicate that the regulation of epsilon globin gene expression may require multiple control elements and that changes in completion or processing of RNA transcripts may contribute to the developmental regulation of the hemoglobin phenotype. Recently we have constructed several transgenic mouse lines expressing fragments of the epsilon globin gene and are testing these elements in this transgenic assay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25021-20 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Sickle Cell Anemia: The Intracellular Polymerization of HbS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
	Eitan Fibach	Visiting Associate	LCB/NIDDK
	Alan N.Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)
BEIB, NIH (R. Chadwick); Pennsylvania Sate University (C. Dong); Paris Consortium (M. Redelsburger, R. Girot, J. Elion); Nippon Medical School, Tokyo, Japan (Drs. N. Uyesaka and S. Hasegawa)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Cell Biology

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.8 PROFESSIONAL: 0.8 OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hemoglobin S polymerization is the primary determinant of the abnormal rheology associated with sickle cell anemia, which is characterized by chronic hemolysis and acute painful crises. Measurements of abnormal cell rheology in sickle cell anemia continue to provide correlations with clinical manifestations although the detailed mechanisms leading to such clinical endpoints are not well understood. The profound effect of hemoglobin S polymerization on cell rheology has given rise to a variety of therapeutic strategies designed to reduce the extent of intracellular polymerization. We have examined the solubility of hemoglobin S mixtures with hemoglobins A, A2 and F at varying oxygen saturations to determine the extent of hemoglobin S polymerization under physiologic conditions. A detailed analysis provides the means to predict the maximum extent of polymerization within the sickle hemoglobin containing erythrocyte. The theoretical model based on experimental data of polymerization of hemoglobin mixtures was used to analyze data obtained from the Parisian Prospective Study on Sickle Cell Disease in children with sickle cell anemia during the first two years of life. These data indicated that 3 of 21 children had a significantly greater predicted polymerization tendency due to early decreases in hemoglobin F. These individuals will be studied prospectively to ascertain the relationship among polymerization tendency and various clinical manifestations of sickle cell disease, as well as the effects of therapy. In our studies of the rheology of sickle cells we have noted that nucleated cells grown in the two-phase culture system will sickle upon deoxygenation. We are studying this phenomenon to learn about the relationship of intracellular polymerization and red cell membrane structure and because this process may occur in the marrow of sickle cell patients and have pathophysiological importance. Lastly, we are continuing our theoretical analyses of the resistance to flow of sickle cells as a function of polymerization, cell density and pulsatile forces to better understand processes in the peripheral blood vessels of patients.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-639

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25025-18 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Mechanism of Antigen-Antibody Interaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hiroshi Taniuchi	Chief, Protein Chemistry and Conformation Section	LCB/NIDDK

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been temporarily suspended.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25058-09 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Human Adult Beta-like Hemoglobin Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Delia C. Tang	IRTA Fellow	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK

COOPERATING UNITS (if any)
Division of Pediatric Hematology, Massachusetts General Hospital, Boston, MA (Dr.David Ebb)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Hematology

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.9 PROFESSIONAL: 0.9 OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been investigating the molecular mechanisms which govern the tissue and developmental stage-specific expression of the adult beta-like globin genes. Our efforts have focused on establishing the combination of trans-acting factors and DNA cis-elements that determines the low level of expression of the adult beta-like globin genes in immature erythroid tissues. It is our hope that elucidating the mechanisms of globin gene regulation will facilitate the manipulate of hemoglobin synthesis in individuals affected by underexpressed or structurally abnormal globin genes. We have employed the K562 human erythroleukemia cell line which has an embryonic-fetal phenotype, which is unique for low level expression of the delta globin gene in the absence of beta globin transcription. This phenotype contrasts markedly with normal erythrocytes from all stages of development, where expression of beta globin far exceeds delta, ranging from a ratio of 75:1 in fetal tissues to 40:1 in normal adults. Using transient transfection assays, we have been exploring the possibilities that both the reversal of normal relative levels of synthesis of delta and beta globin, and the overall low level of expression of the two adult genes in K562 cells are due to the differential binding of *trans*-acting proteins to upstream regulatory regions. DNA fragments from the 5' flanking regions of the delta and beta globin genes was cloned into expression vector containing the CAT or luciferase (LUC) reporter gene, then transiently transfected into the K562 cells. We have clearly demonstrated the existence of a negative transcription element in the -500 to -250bp region of the beta globin gene, consistent with previous studies by Berg (N.A.R., 1989), which identified two negative regulatory elements (BP-1 and BP-2) 5' to the beta globin gene. We suggest that the negative regulatory elements upstream from the beta globin gene play an important role in expression of the adult beta-like globin genes in immature erythroid tissue. We also identified important positive and negative transcription element in the comparable region flanking the delta globin gene. Structural analysis suggests that a potential regulatory element with extensive homology to the putative upstream BP-1 and BP-2 and GATA-1 motifs exists in the delta globin 5' flanking region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25061-20 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Expression of the Human Erythropoietin Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
Others:	Zi-Yao Liu	Visiting Associate	LCB/NIDDK
	Chun Liu	Visiting Fellow	LCB/NIDDK
	Kim Yu	Visiting Fellow	LCB/NIDDK
	Kyung Chin	Microbiologist	LCB/NIDDK

COOPERATING UNITS (if any)
Brown University (Dr. A.A.Anagnostou); Cornell University (Dr. B. Weksler); Columbia University (Dr. F. Constantini); Harvard University (Dr. M. Goldberg)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Molecular Cell Biology Section

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 4.0 PROFESSIONAL: 3.0 OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Erythropoietin (Epo), the primary regulator of the production of erythroid cells, acts by binding to a cell surface receptor (EpoR) on erythroid progenitors. We used deletion analysis and transfection assays to examine the transcription control elements in the 5' flanking region of the human EpoR gene. In erythroid cells most of the transcription activity was contained in a 150 bp promoter fragment with binding sites for transcription factors, AP2, Sp1 and the erythroid specific GATA-1, but lacking a TATA sequence. Mutation analyses indicated that both Sp1 and GATA-1 were required for high level EpoR expression. The EpoR promoter could be transactivated by GATA-1 in K562 erythroid cells and non-erythroid HeLa cells when activity was low. In OCIM1 erythroid cells with high constitutive levels of EpoR, the EpoR promoter activity was high and GATA-1 was able to transactivate only the Sp1 mutated EpoR promoter with low activity. These data suggest that while GATA-1 may be responsible for tissue specific expression of hEpoR, transcription activity depends on coordination between Sp1 and GATA-1 with other cellular factors, including possibly other Sp1-like binding proteins, to provide high-level, tissue-specific expression. We had previously demonstrated that a human EpoR genomic fragment containing about 2 kb of 5' flanking region was able to direct EpoR expression in hematopoietic tissue and in brain. The EpoR promoter and 5' flanking region were linked to the beta-galactosidase gene and assayed in transgenic mice. The 1778 bp 5' flanking region was able to express the reporter gene transiently in the embryonic brain as was observed for the endogenous EpoR gene, while a 150 bp promoter fragment provided reporter gene expression in the adult brain. Neither construct activated reporter gene expression in hematopoietic tissue but did provide expression in testis which is high in GATA-1 but endogenous EpoR expression is silent. These studies indicate that while the 5' flanking region is able to provide some regulation of brain expression, additional 3' sequences are required for activation of EpoR in hematopoietic tissue.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25063-09 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effects of Hydroxyurea on Fetal Hemoglobin Synthesis Beta-Globin Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
Others:	Mary Elizabeth Link	Clinical Nurse Specialist (Research)	LCB/NIDDK
	Sharon Pope	Microbiologist	LCB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Molecular Hematology Section

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 2.5 | PROFESSIONAL: 0.5 | OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have focused on pharmacological augmentation of fetal hemoglobin levels in patients with sickle cell disease and beta-thalasemia. In particular, our group has shown that hydroxyurea (HU), a cell-cycle specific agent that blocks DNA synthesis by inhibiting ribonucleotide reductase, is capable of increasing fetal hemoglobin (HbF) levels in about 75-80% of patients with sickle cell disease by 2-10 fold. These data have been substantiated by others and has been recently culminated by a controlled clinical trial in adult patients in which the HU treated group experienced a significant reduction in the frequency of vaso-occlusive crisis, hospitalization, transfusion requirements and the incidence of acute chest syndrome. An updated analysis of 21 consecutive NIH patients entered on the HU trial, consisting of an initial in-hospital phase, and more prolonged outpatient observation periods has allowed us to draw important conclusions about the response rate, the kinetic aspects of the response to therapy, and the optimal dosage schedules for patients. Although HU treatment of transfusion-dependent β-thalassemia patients has been reported to be disappointing in contrast to the results in sickle cell patients, we have found that some patients with β-thalassemia intermedia, who are not transfusion-dependent may benefit from HU treatment mainly by improving the quality of the newly formed RBC, apparently due to increased HbA levels. In addition, patients double heterozygous for β-thalassemia and HbE display a reciprocal increase in HbF levels with a decline in HbE in response to HU. Thus, not only is the effect of HU likely to be broader than previously appreciated (i.e., including stimulation of βA -synthesis in some patients) but it also may be efficacious in other clinically significant hemoglobinopathies. Current studies are in progress to determine whether particular types of β-thalassemia mutations are more susceptible to γ or β- enhancing effects, to examine the effects of HU on the transcript levels of other globin genes, and to study the effects of HU on transcriptional and post-transcriptional events. Along these line, we have found that HU treatment treatment of a human adult erythroid cell system, which is accompanied by a 2 to 4-fold induction of HbF levels is associated with the loss of binding of three specific transcription factors or factor complexes in the proximal region of the gamma globin gene promoter. Thus studies are underway to confirm the specificity of this observation as well as to further characterize, purify, and eventually clone these factors responsible for fetal globin gene reactivation.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25064-09 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cytogenetic Investigations of Patients with Genetically Determined Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Beverly J. White Director, Cytogenetic Unit LCB/NIDDK

Others:

COOPERATING UNITS (if any)
DDB, NCHGR, (D. Ledbetter and D. Wangsa); DE, NICHD (L. Nelson, J. Baron); LN, NIA (M. Shapiro, D. Murphy, S. Rapoport).

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics (Cytogenetics Unit)

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 1.0 PROFESSIONAL: 1.0 OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The unit conducted collaborative research projects, provided cytogenetic services for NIH patients, and participated in the NIH Medical Genetics Training and College of American Pathologists proficiency testing programs. Case studies were from protocols for short stature, premature ovarian failure (POF), Turner syndrome (TS), mental handicaps, recognized dysmorphic syndromes, neurological diseases, and neoplasia. Detection of aberrations which might lead to mapping of disease genes was a frequent indication for cytogenetic evaluation. Both traditional methods and fluorescent in-situ hybridization (FISH) were used to identify abnormalities.

Numerical and structural sex chromosomal abnormalities were the most frequent variations among 293 new cases. Of all 194 TS patients now screened, 70% were nonmosaic 45,X; the remainder had mosaicism or had X structural abnormalities. Controlled neuropsychological studies in one group of subjects revealed significant abnormalities only in nonmosaics. A controlled longitudinal study comparing mosaic and nonmosaic TS subjects recently reported by other investigators gave similar results. These results will aid in genetic counseling for TS, particularly when detected prenatally, and for establishing prognosis.

Of 187 POF patients now screened, only 3 had abnormal karyotypes. From our previous study of familial POF, our molecular analysis of an X;autosome translocation and literature review, we suspect there are 2 critical regions within Xq for ovarian failure: Xq13.3-q21.1 and Xq26.1-q27. Molecular studies of these regions in familial POF, including a search for Fragile X premutations within the FMR1 gene at Xq27.3, may lead to development of DNA probes for presymptomatic diagnosis.

We are continuing to screen patients with childhood schizophrenia for cytogenetic abnormalities to evaluate an association with deletions of chromosome 22q11.2 reported elsewhere. In a small group of previously karyotyped patients with short stature, screening for uniparental disomy (UPD) of several different autosomes was initiated to determine if certain growth retardation syndromes are associated with this phenomenon. In all of these studies, we interact with the Molecular Cytogenetics Laboratory, DDB, NCHGR.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-[illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25074-06 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A PCR Method for Analysis of Alpha-thalassemia Genotypes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Delia C. Tang	IRTA Fellow	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK

COOPERATING UNITS (if any)
Mahidal University, Bangkok, Thailand (Dr. S. Fucharoen)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Hematology

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The alpha-thalassemias are common genetic disorders that arise from reduced synthesis of the alpha-globin chains of fetal and adult hemoglobins. The normal human alpha-globin gene includes the duplicated alpha-genes 1 and 2. All diploid cells have four alpha-chain genes. In alpha-thalassemia syndromes, alpha-globin synthesis is either diminished or absent due to either deletional or non-deletional abnormalities involving the alpha-globin genes. At present, for a variety of technical, logistic and economic reasons, large-scale carrier screening and appropriate ante-natal detection programs have not been established for the populations in which this syndrome occurs. Here, in our pilot studies, we have successfully established a simple, rapid, sensitive PCR assay for detecting alpha-gene deletion using DNA from fresh blood cell from alpha-thalassemia patients. We have demonstrated that deletion of hemoglobin gene alpha1 or alpha2 could be determined using three sets of primers (two sets for alpha1 and alpha2 gene respectively and one set for beta-actin as internal control) from alpha-thalassemia syndrome patients by the differential PCR. Using this method we have tested hemoglobin alpha genes status from 21 normal subjects and 66 alpha-thalassemia patients. Results clearly demonstrated that different extents of alpha-genes deletion in these patients could be differentiated by measuring the band intensity of the target genes and reference gene after PCR amplification and gel electrophoresis. We have also been studying the PCR mediated color complementary assay, which could serve as a quick, sensitive method to detect alpha-gene deletions omitting gel electrophoresis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25076-06 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of the Epsilon Globin Silencer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Betty Peters	Special Volunteer	LCB/NIDDK
Others:	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
	Alan N.Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS: PROFESSIONAL: OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project report has been combined with ZO1 DK 25016-21LCB.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25077-06 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Globin Gene Expression and the Treatment of Hemoglobinopathies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Shu-Zhen Huang	Senior Staff Fellow	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
	Alan N. Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)
Shanghai Institute of Medical Genetics and Children's Hospital, Shanghai China (Dr. Yi-tao Zeng)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Hematology

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previous studies have shown that hydroxyurea (HU), in addition to its known effects on stimulating gamma-globin chain production, may increase beta-globin chain biosynthesis in some beta+-thalassemia patients with the IVS-2-654 C->T splicing mutation, in which a small amount of normally processed beta-globin mRNA is still produced. As a result, HU-treated patients showed an improvement in the effectiveness of erythropoiesis and alleviation of clinical symptoms. In order to further demonstrate the mechanisms of these changes, we used two-phase liquid human adult erythroid (hAE) cell culture procedure for growing erythroid precursors from two patients with the same IVS-2-654 mutation, coupled with serial RT-PCR mRNA quantitation measurement as well as microglobin chain biosynthetic assays to investigate globin gene expression patterns following HU treatment. The results showed that the level of normally spliced beta-globin mRNA (beta) as compared to the aberrantly spliced form (beta*) was significantly increased in the patients' cultured cells after HU treatment. Globin chain biosynthesis also showed an increase in beta-globin chain production. These results suggest that the increased level of normal beta-globin mRNA may result in part from an improvement in the splicing defect of the IVS-2-654 mutation. They are consistent with our previous findings in beta-thalassemia patients that HU may have a more general role in regulating globin gene expression. Our results are of significance both with respect to developing potential therapies for hemoglobin disorders and gaining insights into the regulation of globin expression.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25078-05 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Globin Expression in an Erythroid Progenitor Culture System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eitan Fibach	Visiting Associate	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
	Alan N. Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)
Department of Hematology, Hadassah Medical School, Jerusalem, Israel (Dr. E. Rachmilewitz); Clinical Hematology Branch, NHLBI (Dr. R. Donahue); Oncogene Sciences Inc., New York (Dr. J. Haley).

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Increased fetal hemoglobin (HbF) in cells of patients with beta-hemoglobinopathies (beta-thalassemia and sickle cell anemia) ameliorates the clinical symptoms of the underlying disease. Recently, several pharmacologic agents have been used to stimulate HbF synthesis; e.g., treatment of patients with hydroxyurea resulted in higher HbF levels and a significant improvement in the clinical symptoms. Yet, there is a considerable interest in other less toxic agents with expanded or higher potential to increase HbF. So far only a handful of agents have been tested, mainly due to the lack of an appropriate experimental system that allows a rapid and accurate determination of the effect on relevant cells. We have recently developed a two-phase liquid culture system for growing erythroid progenitors derived form the peripheral blood of normal individuals and patients with hemoglobinopathies. We showed that the system recapitulates many aspects of erythropoiesis *in vivo* , including stimulation of HbF production by pharmacologic agents. The purpose of our research is to utilize this culture procedure and various biochemical, molecular and immunological methods developed by us lately, to study the regulation of Hb production and for screening of agents for their HbF-stimulating potential with the final objective to produce an efficient, low toxicity therapy. The experiments include optimization of the conditions for stimulation of HbF production, followed by screening of various agents and subsequently studying their mode(s) of action. We have recently found that hemin, and hemin-hydroxyurea combinations, markedly increase HbF in these cells. We are now studying the effects of these drugs in non-human primates. We are also working with Oncogene Sciences, Inc. which has developed a robotic system for screening large numbers of compounds which may affect globin gene transcription. We are testing the compounds which score positively in this system in our culture system. We hope that our studies will facilitate the discovery of new compounds or combinations of drugs that will be clinically useful.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25079-04 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Filterability of Mixtures of Sickle and Normal Erythrocytes - Implication for Exchange Transfusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Setsuo Hasegawa	Special Volunteer	LCB/NIDDK
Others:	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
	Alan N. Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with Z01 DK 25021-20 LCB.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25080-04 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Erythropoietin Receptor Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chun Liu	Visiting Fellow	LCB/NIDDK
Others:	Constance Tom Noguchi	Chief, Molecular Cell Biology Sect.	LCB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project report has been combined with Z01 DK 25061-20 LCB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 25082-04 LCB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Human Erythropoietin Receptor Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kyung Chin	Microbiologist	LCB/NIDDK
Others:	Constance Tom Noguchi	Chief, Molecular Cell Biology Sect.	LCB/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project report has been combined with ZO1 DK 25061-20 LCB.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25084-02 LCB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Transgenic Pig Model for Sickle Cell Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven G. Shapiro	Special Volunteer	LCB/NIDDK
Others:	Constance Tom Noguchi	Chief, Molecular Cell Biology Section	LCB/NIDDK
	Griffin P. Rodgers	Chief, Molecular Hematology Section	LCB/NIDDK
	Alan N. Schechter	Chief	LCB/NIDDK

COOPERATING UNITS (if any)

Veterinary Resources Program, NCRR (Victoria Hampshire, DVM, Chief, Carnivore and Ungulate Unit, Bethesda, MD; Axel Wolff, DVM, Chief, Ungulate Unit, Poolesville, MD); Nextran Inc., Princeton, NJ (Dr. R. Kumar)

LAB/BRANCH Laboratory of Chemical Biology

SECTION Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION NIDDK, Bethesda, MD 20892-1822

TOTAL MAN-YEARS: 0.5 PROFESSIONAL: 0.5 OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to produce a transgenic pig model for sickle cell disease. There is no naturally occurring non-human cognate to the disease and the availability of an animal model would greatly enhance our ability to study the disease and develop new therapies. The pig provides a generally excellent model for human circulatory physiology, with certain aspects of similarity to humans of particular relevance to the model such as analogous responses to vaso-active compounds, a similar response to extracellular hemoglobin, and other similarities in capillary bed density, certain red cell structural properties and the occurrence of cerebrovascular diseases. Previous attempts to make a transgenic mouse model for sickle cell disease have not produced satisfactory symptomology, probably due to differences between murine and human vascular anatomy and physiology.

In order to achieve the high levels of human sickle hemoglobin (HbS) necessary to produce the disease in pigs we are utilizing the most efficient construction for the production of normal human hemoglobin in transgenic full size pigs that has been assembled for synthetic hemoglobin production from the LCR and human alpha and beta genes, and mutagenizing the included adult beta-globin gene to produce the sickle mutation and two additional mutations, Antilles and D Punjab, which promote sickle hemoglobin polymerization. In addition, to facilitate the maintenance and clinical analysis of the transgenic pigs we produce, we are developing a very small strain of pig, the Panepinto miniature swine, as a transgenic system. Using standard protocols developed for the superovulation of full size pigs, we have obtained significant superovulation (up to about 50 ovulations/pig) and recovery of fertilized ova (up to about 20/pig) following natural breeding. Control DNA constructions have been microinjected to develop procedures for the introduction of DNA, surgical reimplantation and maintenance of pregnancy which will be necessary to extend transgenic technology to this more conveniently sized breed. Our ability to understand and treat sickle cell disease should be greatly accelerated by the availability of an accurate transgenic pig model, readily amenable to clinical evaluation.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DK-29001-23-LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular dynamics and vibrational characteristics of membrane assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Ira W. Levin	Research Chemist	LCP-NIDDK
Others:	E. Neil Lewis	Visiting Scientist	LCP-NIDDK
	Linda Kider	IRTA	LCP-NIDDK
	Curtis Meuse	IRTA	LCP-NIDDK
	Steven Barnett	IRTA	LCP-NIDDK

COOPERATING UNITS *(if any)*
C. Huang, School of Medicine, Univ. of VA; J.S. Vincent, Univ. of MD; R. Hendler, NHLBI; D. Lester, FDA; S. Goldstein, NCRR

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued the pioneering of vibrational infrared and Raman spectroscopic imaging techniques, at both the microscopic and macroscopic levels, by advancing the methodology to the mid-infrared spectral region. This spectral interval (2.5μm to 25μm or 4000 cm-1 to 400 cm-1) provides a wealth of vibrational spectroscopic probes for the analysis of systems extending from the biochemical and biophysical disciplines to the analytical chemistry areas. In particular, we have developed and refined a powerful new mid-infrared spectroscopic chemical imaging technique which combines step-scan Fourier transform (FT) Michelson interferometry with indium antimonide (InSb) focal-plane array (FPA) detection. This first time coupling of an infrared focal-plane array detector to an interferometer provides an instrumental multiplex/multichannel advantage, an enormous enhancement for obtaining spectral information. Specifically, the multiple detector elements enable all pixels to be collected simultaneously, while the interferometer portion of the system allows all the spectral frequencies to be measured concurrently. With this method of mid-infrared spectroscopic imaging, the fidelity of the generated spectral images is limited by only the number of pixels defining the FPA detector. Also, only several seconds of detector staring time are required for spectral image acquisition. We believe that this novel, high-definition technique represents the future of infrared chemical imaging analysis, a new discipline within the chemical, biological and material sciences. In addition, this step-scan interferometric approach achieves the ability to perform high-definition mid-infrared imaging in near real-time. Studies have involved the chemical imaging of tissues related to neurotoxicity problems and samples of brain slices of animals treated with a variety of drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29005-21-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Asymmetric Synthesis: Structure, Stereochemistry, and NMR

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.'s	Herman Ziffer	Research Chemist	LCP/NIDDK
Others:	Daniel S. Torok	IRTA Fellow	LCS/NIMH

COOPERATING UNITS (if any)

Dr. Sanford Markey, LCS/NIMH; Dr. H.J.C. Yeh, LAC/NIDDK;
Prof. Steven R. Meshnick, Dept. Epidemiology, Univ. of MI School of Public Health

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A two-step reaction sequence between artemisinin and methanolic ammonia, followed by treatment with Amberlyst 15 yielded 11-aza-artemisinin in 65% yield. When a variety of primary alkyl and heteroaromatic amines were substituted for ammonia in the reaction sequence, a series of N-substituted 11-azaartemisinins were obtained in good yield. By substituting a mixture of sulfuric acid/silica gel for Amberlyst 15 in the reaction sequence, both N-substituted 11-azaartemisinin and N-substituted 10-azadesoxyartemisinin, the expected metabolite, were obtained. *In vitro* test data for the N-substituted 11-azaartemisinins show several are 10 to 25 times more potent antimalarial drugs than artemisinin. The most active derivative, N-(2'-acetaldehydo) 11-azaartemisinin was 4 times more active than artemisinin *in vivo*. The reaction scheme is shown below.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29008-24-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electric and molecular structure investigation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Ruth McDiarmid	Research Chemist	LCP-NIDDK
Others:	Xing Xing	Visiting Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

Bjoern Roos, Chem. Centre, Lund, Sweden; M. Merchan, Univ. of Valencia, Spain

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several liquid chromatography/mass spectrometry (LC/MS) inlet system for our supersonic jet - molecular beam - time of flight mass and optical spectrometer have been designed and tested with methanol solutions of indole, tryptophan, or tryptamine. The results obtained with indole demonstrate that bare indole and indole - methanol (1-7 methanols) clusters are generated. The former have much stronger and sharper optical spectra than the latter. Tryptophan and tryptamine have been volatilized from the methanol solution but as yet have not been observed spectrometrically.

The far UV spectrum of acetone has been studied by 3-photon REMPI and PA spectroscopies and, in collaboration, theoretically. All results were combined to locate and partially characterize the pi -> pi* transition of acetone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29010-23-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dynamics of Proteins and Studies on Sickle Cell Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	William A. Eaton	Medical Officer	LCP-NIDDK
Others:	James Hofrichter	Research Chemist	LCP-NIDDK
	Eric Henry	Research Physicist	LCP-NIDDK
	Garrott Christoph	Physicist	LCP-NIDDK
	Stephen Hagen	Staff Fellow	LCP-NIDDK
	Chi-Kin Chan	Visiting Associate	LCP-NIDDK
	Yi Hu	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nanosecond laser temperature-jump and rapid chemical dilution of chemical denaturants using a new ultrafast continuous flow method are being developed and applied to the problem of describing the earliest events in protein folding. The laser temperature jump will be used to initiate the folding of cold-denatured proteins. In the ultrafast flow method the time course of protein folding is monitored by the fluorescence of the lone tryptophan (W59) which decreases as the protein folds due to quenching by the heme. The decay of the fluorescence, as well as the tryptophan-heme distance calculated from the quantum yield, are highly non-exponential extending from tens of microseconds to seconds. These results are interpreted in terms of energy landscape theory as "downhill run" to the native conformation with an effective diffusion constant decreases as the protein becomes more compact.

Using time-resolved absorption spectroscopy to monitor the kinetics of geminate rebinding and conformational changes in myoglobin we have shown that the solvent viscosity, rather than the low temperature, is primarily responsible for the trapping of conformational substrates. This is demonstrated by placing myoglobin in a glass at room temperature and observing inhomogeneous reaction kinetics. The high solvent viscosity dramatically slows the rate of crossing the energy barriers separating substrates, and also suppresses any change in the average protein conformation following ligand dissociation.

A simple model explain stretched exponential conformational relaxation in proteins has been developed. The basic idea of the model is that non-exponential relaxation results from transitions among two groups of protein conformational substrates connected by transition states of a single energy.

We have measured oxygen binding curves of single crystals of hemoglobin in which the iron in the α subunits has been replaced by the non-oxygen binding nickel(II). These results provide additional strong support for the basic idea of allosteric theory that hemoglobin in the *T* quaternary conformation binds oxygen non-cooperatively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29011-24 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
The physics and chemistry of photoreception

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: William A. Hagins, Medical Officer, LCP-NIDDK
Others: S. Yoshikami, Research Biologist, LCP-NIDDK
P. Ross, Research Chemist, LCP-NIDDK
Mark Vivino, Computer Systems Analyst, CSL-DCRT

COOPERATING UNITS *(If any)*
Sudhir Sahu and Kalpana Mani, Student Volunteers, Thomas Jefferson High School, Alexandria, VA.

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Membrane Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Members of a family of fluorescent calcium-sensitive indicator dyes including Calcium Green, Calcium Orange, Calcium Crimson, Flo-3 and Rhod-2 have been transferred to retinal rods of frogs by the silylated-dye method described in previous reports in 1993-4. Dye levels exceeding 1 micromolar in the outer segments of rods have been achieved with one hour incubations. The dyes partially emerge from the labeled cells when the cell membranes are ruptured osmotically.

2. Studies by image-intensified fluorescence microscopy of the responses of the dyes to calcium ions show that the indicators are almost completely bound to the rod disk membranes and are almost totally insensitive to changes in calcium ion activity in the bathing solutions. Analysis of the polarization of the fluorescence emitted by the dyes Calcium Green, Calcium Crimson, and Calcium Orange show that the E-vectors of the emitting oscillators are parallel to the surfaces of the disk membranes, while that of the membrane responsive fluorophore N,N' Bis(dansyl cystine) is parallel to the fatty acid side chains in the membranes. (Yoshikami, Hagins, Sahu)

3. A family of precursors for chelation of divalent ions, including Ca++, have been synthesized with functional groups ready for coupling to fluorescent chromophores. The aim is to produce a group of ned Calcium-sensitive fluorophores that are not inactivated by binding to cell organelles as are the above-studied dyes available commercially. A total of eight chelators have been made and studies of the coupling reactions are under way. (Hagins, Mani)

4. A pyroelectric photocalorimeter that uses evanescent-waves from stripped optical fibers to introduce photo excitation into small fluid samples is under construction. (Hagins)

5. A pyroelectric calorimeter with improved signal-to-noise properties is under construction forstudying phototransduction in frog retinal rods at very low light intensities. Improvements will make it possible to measure heat bursts from nucleotide hydrolysis within 1-2 minutes after the tissue is introduced into the calorimeter. (Yoshikami, Hagins)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29016-18 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Macromolecular dynamics and assembly reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: James Hofrichter, Research Chemist, LCP-NIDDK
Others: Garrott Christoph, Physicist, LCP-NIDDK
Eric Henry, Research Chemist, LCP-NIDDK
William A. Eaton, Medical Officer, LCP-NIDDK
Steve Hagen, Staff Fellow, LCP-NIDDK
Chi-Kin Chan, Visiting Associate, LCP-NIDDK
Yi Hu, Visiting Associate, LCP-NIDDK
Peggy Thompson, IRTA, LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Laser Biophysics and Spectroscopy

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Time-resolved absorption and fluorescence spectroscopy are used to study the dynamics of protein structural changes subsequent to rapid mixing or excitation with short laser pulses. Molecular models for the protein dynamics are used to fit and interpret the measured data.

A. We have succeeded in implementing two new techniques which can be used to study the kinetics of a wide variety of processes, a rapid mixing technique which, in principle, is capable of initiating reactions within 10 microseconds and a laser temperature jump experiment in which water is heated by an infrared laser pulse.

B. The dynamics of short-lived states on the folding pathway of cytochrome c have been investigated using continuous-flow mixing and fluorescence quenching to monitor the tryptophan-heme distance. The most rapid phase observed occurs at about 100 microseconds, followed by a decay in fluorescence intensity which includes at least 4 additional exponential relaxations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29016-18 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters of less. Title must fit on one line between the borders.)
Macromolecular dynamics and assembly reactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
P.I.: James Hofrichter, Research Chemist, LCP-NIDDK
Others: Garrott Christoph, Physicist, LCP-NIDDK
Eric Henry, Research Chemist, LCP-NIDDK
William A. Eaton, Medical Officer, LCP-NIDDK
Steve Hagen, Staff Fellow, LCP-NIDDK
Chi-Kin Chan, Visiting Associate, LCP-NIDDK
Yi Hu, Visiting Associate, LCP-NIDDK
Peggy Thompson, IRTA, LCP-NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Laser Biophysics and Spectroscopy

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Time-resolved absorption and fluorescence spectroscopy are used to study the dynamics of protein structural changes subsequent to rapid mixing or excitation with short laser pulses. Molecular models for the protein dynamics are used to fit and interpret the measured data.

A. We have succeeded in implementing two new techniques which can be used to study the kinetics of a wide variety of processes, a rapid mixing technique which, in principle, is capable of initiating reactions within 10 microseconds and a laser temperature jump experiment in which water is heated by an infrared laser pulse.

B. The dynamics of short-lived states on the folding pathway of cytochrome c have been investigated using continuous-flow mixing and fluorescence quenching to monitor the tryptophan-heme distance. The most rapid phase observed occurs at about 100 microseconds, followed by a decay in fluorescence intensity which includes at least 4 additional exponential relaxations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29019-15-LCP

OD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Theoretical studies on the dynamical aspects of macromolecular function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Szabo	Research Chemist	LCP-NIDDK
Others:	X. Zhou	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*
A. Gronenborn and M. Clore, LCP-NIDDK; D. Torchia, NIDR; and L. Kay, University of Toronto.

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Theoretical Biophysical Chemistry Section

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

HECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A molecular theory for the rate of nonadiabatic electron transfer was developed and its relation to classical Marcus theory was established. The rate was found to be determined by the probability density of the energy gap, which is defined as the instantaneous change in solvation energy upon moving an electron from the donor to the acceptor. It was shown how this probability density can be obtained from the free energies of transferring varying amounts of charge between the donor and acceptor (as specified by a charging parameter). A simple algorithm was proposed for calculating these free-energy changes (and hence the energy gap probability density) from computer simulations on just three states: the reactant, the product, and an "anti" product formed by transferring a positive unit charge from the donor to the acceptor. The Marcus relation (i.e., the activation energy as a parabolic function of the free-energy change of reaction) was derived in a way that clearly shows that it is a good approximation in the normal region even when the solvent response is significantly nonlinear. A simple generalization of this relation, in which the activation energy is given by parabolic functions with different curvatures in the normal and inverted regions, was proposed. A novel method has been developed to analyze NMR relaxation experiments on unfolded proteins. It was shown how the spectral density function describing the dynamics of amide bond vectors can be determined at specific frequencies using N15 relaxation parameters alone. Experimental data on the folded and unfolded forms of a small protein was used to establish the validity of this procedure. A continuum model of electrostatic interactions in proteins has been used to analyze the energetics of a positionally disordered water molecule within an apparently hydrophobic cavity of a protein, as has recently been found experimentally using NMR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29020-10-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear magnetic resonance: new methods and molecular structure determination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Ad Bax Visiting Scientist LCP-NIDDK

Others:
Rolf Tschudin (Electronics Engineer)
Andy Wang (Special Volunteer)
David Live (Guest Worker)
Nico Tjandra (Special Volunteer)
Jin-shan Hu (Special Volunteer)
Frank Delaglio (Special Expert)
Stephen Grzesiek (Visiting Assoc)
Hitoshi Kuboniwa (Special Volun.)
John Marquardt (Special Volun.)

COOPERATING UNITS *(if any)*

Marius Clore & Angela Gronenborn, LCP-NIDDK; Dennis Torchia, BRB-NIDR; Claude Klee, LB-NCI; Paul Wingfield and Stephen Stahl, Protein Expression Laboratory, OD

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Biophysical Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	7.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New methods have been developed for measurement of homo- and heteronuclear J couplings in isotopically enriched proteins. These methods permit these J couplings to be measured at an unusually high precision of *ca* 0.1 Hz. Nearly forty years ago, Karplus demonstrated an empirical relation between the size of the three-bond J coupling and the intervening dihedral angle. Besides the dihedral angle, J couplings are also known to be influenced by a number of other factors, such as substituent effects, electric field gradients, and bond strain. In proteins, there are four three-bond J couplings that describe the backbone angle ϕ. All four J couplings have been measured for most of the amino acid residues in a small model protein, ubiquitin. Comparison of the measured J couplings with the ϕ angles calculated from the X-ray structure of this protein allowed reparametrization of the "Karplus relationships" which previously had been based on the measurement of J couplings in constrained small model compounds. Although qualitatively similar, the new parametrizations deviate considerably from the commonly used literature values. Concerted use of all four J couplings for a given residue allows the ϕ angle to be determined at high precision. Results indicate that the uncertainty in the NMR-derived ϕ angle is *ca* 2°, which compares favorably with the uncertainty of *ca* 3.2° observed for the ϕ angle in the X-ray structure of basic pancreatic trypsin inhibitor, solved at 1-Å resolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29021-10-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conformation and dynamics of biological molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Eric Henry	Research Physicist	LCP-NIDDK
Others:	William Eaton	Medical Officer	LCP-NIDDK
	Colleen Jones	Staff Fellow	LCP-NIDDK
	James Hofrichter	Research Chemist	LCP-NIDDK

COOPERATING UNITS *(if any)*

Andrea Mozzarelli, University of Parma

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Biophysical Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used kinetic modelling to analyze sets of time-resolved spectroscopic data measured for hemoglobin over a range of fractional ligand photodissociation. The model used is a kinetic formulation of the two-state allosteric model extended in a minimal fashion to incorporate the geminate ligand rebinding and tertiary conformational changes detected in the measurements. The model incorporates three spectroscopically distinguishable states of unliganded hemoglobin subunits, and the kinetic description includes a stretched-exponential form for the tertiary conformational relaxation as well as a linear free energy relation connecting the quaternary transition rates for tetramers with different numbers of bound ligands. Fits using this model produce spectra for the three unliganded species which resemble the spectrum of deoxyhemoglobin and differ in a manner that is consistent with the ligand binding affinities of the species. The fits predict that both tertiary and quaternary conformational relaxations have a marked effect on the geminate ligand rebinding rate. We have also shown from measurements of polarized absorption spectra of metal-hybrid hemoglobin crystals that the ratio of the affinities of the α and ß subunits of hemoglobin in the crystal is even less than the factor of 3-5 estimated in earlier work.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29022-07 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Studies of AIDS proteins by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Angela M. Gronenborn, Visiting Scientist, LCP-NIDDK
G. Marius Clore, Visiting Scientist, LCP-NIDDK
Ad Bax, Visiting Scientist, LCP-NIDDK
Others: Robert Clubb, Guest Researcher, LCP-NIDDK; James Ernst, Pre-Irta, LCP-NIDDK, Daniel Garrett, Irta Fellow, LCP_NIDDK; Sephan Grzesiek, Visiting Associate, LCP-NIDDK, Patricia Lodi, Guest Researcher, LCP-NIDDK; John Kuszewski, Pre-Irta, LCP-NIDDK, Jim Omichinski, Staff Fellow, LCP-NIDDK; Milton Werner, Irta Fellow, LCP-NIDDK

COOPERATING UNITS *(if any)*
LMB, NIDDK (Robert Craigie); NCI (Ettore Appella, K. Sakaguchi); Protein Expression Laboratory (Paul Wingfield, Stephan Stahl)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Sections on Structural Biology, Protein NMR Spectroscopy, and Biophysical NMR

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.6	PROFESSIONAL: 2.6	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work has been carried out on a number of structural problems related to proteins derived from the HIV-1 virus. These include the DNA binding domain of the integrase, the NEF protein and the p7 nucleocapsid protein. The high resolution three-dimensional structure of the dimeric DNA binding domain of HIV-1 integrase has been determined. In addition, work on complexes of a double zinc binding domain of the transcription factor GATA-1 and the ETS-1 transcription factor with their cognate DNA target sites are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29023-05 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Determination of Three-Dimensional Structures of Macromolecules in Solution by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: G. Marius Clore, Visiting Scientist, LCP-NIDDK
Angela M. Gronenborn, Visiting Scientist, LCP-NIDDK
Others: Jim Omichinski, Staff Fellow, LCP-NIDDK; John Kuszewski, Predoctoral Irta, LCP-NIDDK; Teresa Strzelecka, Visiting Associate, LCP-NIDDK; Patricia Lodi, Special Volunteer, LCP-NIDDK; Jun Qin, Visiting Fellow, LCP-NIDDK; Kirsten Frank, Guest Researcher, LCP-NIDDK; Jeff Huth, Milton Werner, William Kennedy, Daniel Garrett, and Robert Clubb, Irta Fellows, LCP-NIDDK

COOPERATING UNITS *(if any)*
LCP, NIDDK (A. Bax) ; LMB, NIDDK (G. Felsenfeld, K. Mizuuchi, R. Craigie); NCI (E. Appella, D. Covell); NHLBI (A. Peterkofski); Yale (K. Williams); Johns Hopkins Univ. (G. Makhatadze); Univ. of NV-Reno (R. Harrington).

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Protein NMR Spectroscopy and Section of Structural Biology

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	4.8	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work in this laboratory has been focused on the determination of three-dimensional structures of larger proteins in solution by NMR, with a particular emphasis on protein-protein, protein-ligand and protein-DNA complexes. A considerable effort has been placed on the development of three- and four-dimensional heteronuclear NMR to extend the application of NMR as a method for determining three-dimensional structures of proteins in solution beyond the limits of conventional two-dimensional NMR (~100 residues) to molecules in the 150- to 400-residue range.

High resolution solution structures of a number of proteins have been determined. These include the oligoomerization domain of p53, a complex of human thioredoxin with its target site from the transcription factor NFkB, the DNA binding domain of Mu transposase, the DNA binding domain of HIV-1 integrase, and the specific complex of human SRY (the male sex determining factor) with DNA. The latter provides the molecular basis for understanding mutations in SRY that lead to 46X,Y sex reversal. Studies on protein hydration have revealed the presence of conformationally disordered water within a large 100-200_3 internal hydrophobic cavity of interleukin-1b.

In addition, methodological advances have been made with regard to refinement of NMR structures, including the use of coupling constants, carbon chemical shifts and proton chemical shifts directly into the target functions employed in refinement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 29025-06 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Investigations of Macromolecular Structures and Dynamics in Solution by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Angela M. Gronenborn, Visiting Scientist, LCP-NIDDK
G. Marius Clore, Visiting Scientist, LCP-NIDDK
Others: Jim Omichinski, Staff Fellow, LCP-NIDDK; John Kuszewski, Predoctoral Irta, LCP-NIDDK; Teresa Strzelecka, Visiting Associate, LCP-NIDDK; Patricia Lodi, Special Volunteer, LCP-NIDDK; Jun Qin, Visiting Fellow, LCP-NIDDK; Jeff Huth, Milton Werner, William Kennedy, Daniel Garrett, and Robert Clubb, Irta Fellows, LCP-NIDDK

COOPERATING UNITS *(if any)*
LCP, NIDDK (A. Bax) ; LMB, NIDDK (G. Felsenfeld, K. Mizuuchi, R. Craigie); NCI (E. Appella, D. Covell); NHLBI (A. Peterkofski); Yale (K. Williams); Johns Hopkins Univ. (G. Makhatadze); Univ. of NV-Reno (R. Harrington).

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section of Structural Biology and Section on Protein Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.8	PROFESSIONAL: 4.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the overall research in this laboratory is centered on achieving as complete a description as possible for the structures of peptides, proteins, nucleic acids and their complexes in solution, principally by NMR spectroscopy. At present particular emphasis is being placed on developing approaches which allow the investigation of larger and complex systems as well as increase the precision with which these solution structures can be obtained, studies aimed at correlating structure and function, and experiments aimed at investigating protein folding.

Structures for several proteins have been determined and analyzed. These include the oligomerization domain of p53, a complex of human thioredoxin with its target site from the transcription factor NFkB, the DNA binding domain of Mu transposas, the DNA binding domain of HIV-1 integrase, and the specific complex of human SRY (the male sex determining factor) with DNA. The latter provides the molecular basis for understanding mutations in SRY that lead to 46X,Y sex reversal. Studies on protein hydration have revealed the presence of conformationally disordered water within a large 100-120_3 internal hydrophobic cavity of interleukin-1b.

In addition, work on the unfolded state of Streptococcal Protein G is continuing and the structure and dynamics of the unfolded state have been characterized by NMR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 29025-06 LCP

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigations of Macromolecular Structures and Dynamics in Solution by NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Angela M. Gronenborn, Visiting Scientist, LCP-NIDDK
G. Marius Clore, Visiting Scientist, LCP-NIDDK
Others: Jim Omichinski, Staff Fellow, LCP-NIDDK; John Kuszewski, Predoctoral Irta, LCP-NIDDK; Teresa Strzelecka, Visiting Associate, LCP-NIDDK; Patricia Lodi, Special Volunteer, LCP-NIDDK; Jun Qin, Visiting Fellow, LCP-NIDDK; Jeff Huth, Milton Werner, William Kennedy, Daniel Garrett, and Robert Clubb, Irta Fellows, LCP-NIDDK

COOPERATING UNITS *(if any)*
LCP, NIDDK (A. Bax) ; LMB, NIDDK (G. Felsenfeld, K. Mizuuchi, R. Craigie); NCI (E. Appella, D. Covell); NHLBI (A. Peterkofski); Yale (K. Williams); Johns Hopkins Univ. (G. Makhatadze); Univ. of NV-Reno (R. Harrington).

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section of Structural Biology and Section on Protein Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.8	PROFESSIONAL: 4.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the overall research in this laboratory is centered on achieving as complete a description as possible for the structures of peptides, proteins, nucleic acids and their complexes in solution, principally by NMR spectroscopy. At present particular emphasis is being placed on developing approaches which allow the investigation of larger and complex systems as well as increase the precision with which these solution structures can be obtained, studies aimed at correlating structure and function, and experiments aimed at investigating protein folding.

Structures for several proteins have been determined and analyzed. These include the oligomerization domain of p53, a complex of human thioredoxin with its target site from the transcription factor NFkB, the DNA binding domain of Mu transposas, the DNA binding domain of HIV-1 integrase, and the specific complex of human SRY (the male sex determining factor) with DNA. The latter provides the molecular basis for understanding mutations in SRY that lead to 46X,Y sex reversal. Studies on protein hydration have revealed the presence of conformationally disordered water within a large 100-120_3 internal hydrophobic cavity of interleukin-1b.

In addition, work on the unfolded state of Streptococcal Protein G is continuing and the structure and dynamics of the unfolded state have been characterized by NMR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29026-07-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR and other spectroscopic studies of molecular structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Edwin D. Becker	Research Chemist	LCP-NIDDK

COOPERATING UNITS *(if any)*

Sophisticated Instruments Facility, Indian Institute of Science, Bangalore, India
Chemistry Department, Georgetown University, Washington, DC

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NMR investigations with liquid crystals are being used to study weak molecular interactions and to provide information on the degree of ordering of molecules dissolved in a liquid crystal solvent. Samples have been studied in nematic solvents while being spun at the "magic angle." With natural abundance carbon-13 NMR, the relative intensities of the spinning sidebands have been shown to provide information on the sign of the order parameter at each carbon atom in the molecule.

We are continuing to explore the mechanism and potential applications of the effect of isotopic substitution on NMR chemical shifts of nuclei that are several bonds away from the site of substitution. Studies of a number of differently substituted binuclear aromatic molecules (including *cis*- and *trans*-stilbenes, *trans*-azobenzene, diphenyl-acetylene and diphenylethane) were previously reported to show isotope-induced changes in carbon-13 chemical shifts through as many as 10 chemical bonds and as small as 0.64 parts per billion. The relation of the observations to details of molecular structure is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29027-07-LCP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical studies of dynamical processes in chemical physics and biophysics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Robert Zwanzig Research Chemist LCP-NIDDK

Others:

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Theoretical Biophysics Section

INSTITUTE AND LOCATION

NIH, NIDDK. Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	**PROFESSIONAL:** 1.0	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One project dealt with "globally connected master equations", which are schemes for treating the kinetics of very complex systems, for example proteins. Another project was concerned with the topographical roughness of potential energy functions in many body systems. This led to a paper critical of certain methods that have been proposed to find the distribution of barrier heights. A third project under way is a continuing critical study of proposed theories of protein folding. The goal is to find a theory that is both simple and in agreement with what is known about the folding process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29028-05-LCP

PERIOD COVERED

October 1994 through September 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Free energy conversion in biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Yi-der Chen	Research Chemist	LCP-NIDDK
Others:	Robert J. Rubin	Special Volunteer	LCP-NIDDK

COOPERATING UNITS *(if any)*

T. Y. Tsong, Dept. of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong.

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Theoretical Biophysics Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general aim of this project is to study the molecular mechanisms of free energy transduction in biology, such as in muscle contraction, kinesin movement, active transport, flagellar rotation, etc. During this period, our work was concentrated on the question of how a biological motor (such as myosin and kinesin) would carry out the repeated *directional* movements on a periodic biopolymer (such as actin and microtubule) by utilizing the free energy of ATP hydrolysis. These repeated directional movements are implicated in the theory of muscle contraction. Without going into complicated biological systems, we examined the simplest case: The chemically driven motility of a brownian particle. We showed with a simple model that an enzymatic brownian particle when coupled with a chemical reaction could move unidirectionally on a spatially periodic and asymmetric potential, if the intermediate states in the catalytic cycle interacted with the potential differentially and that the chemical reaction was out of equilibrium. The general principle of the model could be tested experimentally and should prove useful in biomolecular separation. The model can also be generalized and used to study the motility of single biological motors in *in vitro* experiments, where brownian motion may play an important role.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31100-30 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	T. Spande	Research Chemist	LBC, NIDDK
	H.M. Garraffo	Visiting Associate	LBC, NIDDK
	X.-F. Pei	Visiting Fellow	LBC, NIDDK
	B. Badio	IRTA	LBC, NIDDK
	P. Jain	Visiting Fellow	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK

COOPERATING UNITS *(if any)*

T. Tokuyama, Osaka City U., Japan; C.W. Myers, AMNH, NYC; E. Gros, U. Buenos Aires, Argentina; J. Cover, NAIB, Baltimore, MD; M. Andriantsiferana, Madagascar; A.S. Rand, STRI, Panama, Panama; T.H. Jones VMI, Lexington, VA; M. Lazdunski, CNRS, Valbonne, France.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	3.9	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Natural products provide a wide range of biologically active agents, many of which have unique profiles of pharmacological activity and therapeutic potential. Over three hundred alkaloids have been identified in extracts from amphibian skins. These include batrachotoxins, which are potent activators of sodium channels, histrionicotoxins, which are noncompetitive blockers of nicotinic receptor channel complexes and potassium channels, pumiliotoxins, which have myotonic and cardiotonic activity due to effects on sodium channels, and epibatidine, an extremely potent and selective nicotinic agonist with remarkable antinociceptive activity. Further alkaloids include 2,5-disubstituted decahydroquinolines, 3,5-disubstituted pyrrolizidines , 3,5-disubstituted indolizidines, 5,8-disubstituted indolizidines, 1,4-disubstituted and 4,6-disubstituted quinolizidines, the pumiliotoxin-homopumiliotoxin-allopumiliotoxin class, and a variety of tricyclic alkaloids, including pyrrolizidine oximes, pseudophrynamines, cyclopentaquinolizidines and coccinellines. Most of these alkaloids have been detected and characterized from neotropical dendrobatid frogs. Pumiliotoxins also occur in one genus of Australian myobatrachid frogs, in one genus of South American bufonid toads and in one genus of Madagascan mantellid frogs. The myobatrachid frogs also contain a unique class of physostigmine-like alkaloids, the pseudophrynamines, while the bufonid toads and the mantellid frogs contain, in addition to pumiliotoxins, a wide array of pyrrolizidines indolizidines, quinolizidines, decahydroquinolines and tricyclic alkaloids. Batrachotoxins occur in a New Guinea bird and alkaloids have been detected in two other birds. Structures of a number of further alkaloids are currently under investigation. Pyrrolizidine oximes have been shown to be nicotinic blockers and pseudophrynamine to be a sodium channel blocker. The antinociceptive activity of epibatidine has been further defined and proposed to be due to agonist activity at $\alpha_3\beta_4$ and $\alpha_4\beta_2$ nicotinic receptors. Calcium channel activity is involved in the antinociceptive effects of epibatidine. Analogs of epibatidine are being synthesized. It appear all frog skin alkaloids have a dietary origin, being taken up and sequestered unchanged into skin glands. Ants, beetles and millipedes appear to be the source of certain frog skin alkaloids. The origin of the batrachotoxins, histrionicotoxins, pumiliotoxins, decahydroquinolines and epibatidines found in frog skin remains a mystery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31101-26LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Metabolism of Biogenic Amines and Related Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C. R. Creveling, Research Chemist, LBC, NIDDK
Others: J.W. Daly, Chief, LBC, NIDDK
K.L. Kirk, Research Chemist, LBC, NIDDK

COOPERATING UNITS *(if any)*

Weisz, J., U.Penn., Hershey, PA.; Inoue, Matsumoto Dent. Col. Nagano, Japan.; Nagatsu, U. Nagoya, Japan., Liehr, J., U. Texas, Galveston, TX., Weinshilboum, R., Mayo Clinic, Rochester, MI., W. Dixon, U. Kansas, Lawrence, KA.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemistry, physiology, and pharmacology of biogenic amines, catecholsteroids, amino acid precursors, metabolic products, various synthetic derivatives as well as related biosynthetic and metabolic enzymes have been investigated. The primary area of study concerns various aspects of catechol-0-methyltransferase (COMT)including: immunohistochemical localization of COMT as well as catecholamine levels in the dental pulp of rat and man; the immunohistochemical localization of the tyrosine hydroxylase, aromatic amino acid decarboxylase, and L-DOPA in the dental pulp of rat and man; the effect of RU486 on the induction of COMT in the pregnant rat uterus; the effect on COMT inhibition during day 0 to day 3 of pregnancy on implantation in the rat; a study of the temporal relation between the appearance of COMT, progesterone, and estrogen receptors in the luminal epithelium of the pregnant rat; the localization of COMT in estrogen-induced tumors of the hamster kidney is in progress; a survey of the correlation of "low" red blood cell-COMT activity with the incidence of adenocarcinomas and other evidences of neoplastic activity in estrogen sensitive tissue sites in women has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31102-24 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ion Channels, Receptors and Second Messengers in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	B. Badio	IRTA	LBC, NIDDK
	Y. Shin	IRTA	LBC, NIDDK
	H.M. Garraffo	Visiting Associate	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK

COOPERATING UNITS *(if any)*

T. Yasumoto, Tohoku Univ., Sendai, Japan; J. Adelman, Vollum Institute, Portland, OR; F. Gusovsky, Eisai Res. Inst., Andover, MA.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium, sodium, potassium, and magnesium ions after translocation through ion channels or by transport proteins can cause activation of release processes, contractile proteins, adenylate and guanylate cyclase, phosphodiesterases, protein kinases, phospholipases, ATPases and other enzymes. Receptors of various types and various toxins serve to modulate ion channels and generation of second messengers including cyclic nucleotides, diacylglycerides, arachidonic acid and phosphatidic acid. Modulatory interactions or "cross-talk" occurs both between the second messenger systems and with the ion transport systems. Maitotoxin, a marine polyether increases phospholipid breakdown, leading to inositol phosphate and diacyclglyceride production. The primary site of action of maitotoxin appears to be a calcium channel. Maitotoxin-elicited influx of calcium was blocked by imidazoles (SKF 96365, econazole, miconazole, clotrimazole) that also effectively block the so-called calcium release-activated calcium (CRAC) channels. Such imidazoles have proven to be the only class of compounds that block CRAC channels, but there were many other compounds that effectively blocked maitotoxin-elicited influx of calcium. Some of the more potent versus maitotoxin were fluspirilene, penfluridol, loperamide, RMI 1233OA, and proadifen. Thus, maitotoxin does not appear to act primarily through effects on CRAC channels. Loperamide was found to be unique in enhancing influx of calcium via CRAC channels that had been activated either by a receptor-mediated release of intracellular calcium or by thapsigargin-mediated depletion of intracellular pools of calcium. The stimulatory effects of loperamide have been demonstrated in several cell lines. A series of quaternary amines have been prepared to probe the structural requisites for blockade of potassium channels by the classical blocker tetraethylammonium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK31104-27 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. M. Jerina	Section Chief	LBC, NIDDK
Others:	J. M. Sayer	Research Chemist	LBC, NIDDK
	H. Yagi	Visiting Scientist	LBC, NIDDK
	N. T. Nashed	Research Chemist	LBC, NIDDK
	N. Ya	IRTA Fellow	LBC, NIDDK
	C. Liang	IRTA Fellow	LBC, NIDDK
	Z. Zhong	IRTA Fellow	LBC, NIDDK
	A. M. Cheh, H. D. Mah	Special Volunteers	LBC, NIDDK

COOPERATING UNITS *(if any)*

A. Conney, Rutgers U.; W. Levin, Roche Res. Center (Nutley, NJ); D. Boyd, Queen's U. Belfast (N. Ireland); P. LeBreton, U. IL (Chicago); A. Dipple, FCRDC, Frederick, MD; D. Whalen, U. MD (Baltimore); H. Yeh, LAC, NIDDK; D. Gorenstein, Purdue U.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Oxidation Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892-0820

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	6	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NMR solution structures have been described for 3 oligonucleotide duplexes modified at N-6 of the central deoxyadenosine (dA) residue in the sequence 5'-GGTCA*CGAG-3' by trans addition to C-10 of isomeric 7,8-diol 9,10-epoxide metabolites of benzo[a]pyrene (BPDE). These are two nonanucleotide duplexes with deoxyguanosine (dG) mismatches opposite dA adducts derived from the enantiomers (+)-BPDE-2 and (-)-BPDE-2 [diastereomer with the benzylic hydroxyl group and epoxide oxygen trans], and an undecanucleotide duplex with the same internal sequence and a T residue opposite a dA adduct derived from (-)-BPDE-1 [diastereomer with the benzylic hydroxyl group and epoxide oxygen cis]. Major findings are: i) The aromatic portion of the hydrocarbon is intercalated into the DNA helix, and lies toward the 5'-end of the modified DNA strand when the absolute configuration at C-10 is R, and toward the 3'-end when the configuration at C-10 is S; ii) In the oligomer derived from (-)-BPDE-2, the glycosidic bond of the modified dA has a normal, anti conformation, and the mismatched G is rotated out of the DNA helix, whereas the glycosidic bond of the (+)-BPDE-2 dA adduct has an unusual syn conformation, and the A is hydrogen bonded to the mismatched G; iii) The BPDE-1 dA adduct in the modified undecamer maintains normal hydrogen bonding with a complementary T; iv) Both duplexes with a mismatched G opposite a modified A exhibit a major and a minor conformation by NMR, in contrast to oligonucleotides containing trans BPDE-2 adducts at the exocyclic N-2 of dG, which were observed to be conformationally homogeneous. Fluorescence decay profiles of the two nonanucleotide duplexes containing dA adducts, as well as two undecanucleotides containing trans dG adducts from (+)-and (-)-BPDE-2, are similar, and exhibit two short-lived components (≥95% of total intensity) and one minor, long-lived component. Since the dG modified oligonucleotides show only one conformation by NMR, their two fluorescence lifetimes may result from conformations that interconvert rapidly on the NMR time scale. In 18mer templates, trans dA adducts from BPDEs block primer extension by DNA polymerases to an extent that depends both on the specific enzyme and the absolute configuration of the adduct.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK31106-08 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanistic Enzymology of HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D. M. Jerina	Section Chief	LBC, NIDDK
Others:	J. M. Sayer	Research Chemist	LBC, NIDDK
	N. T. Nashed	Research Chemist	LBC, NIDDK
	M. T. Haber	Adjunct Researcher	LBC, NIDDK
	N. Ya	IRTA	LBC, NIDDK

COOPERATING UNITS *(if any)*

J. M. Louis and A. R. Kimmel, LCDB, NIDDK; K. D. Parris, LMB, NIDDK

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Oxidation Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892-0820

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms of action of the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) as well as other DNA polymerizing enzymes are being investigated with the goal of developing specific inhibitors for RT. We have previously reported steady-state kinetics for the incorporation of a single nucleotide residue derived from thymidine or 4-thiothymidine triphosphate (TTP or 4S-TTP respectively) into a primer, TCGCAGCCG, annealed to the DNA template, AAACCCTTGGACGGCTGCGA, catalyzed by the Klenow fragment of Escherichia coli DNA polymerase I. These results have now been supplemented by single turnover studies using stopped flow spectrophotometry. Measurements (340 nm) of reaction progress on a millisecond time scale for periods up to 2 s were made at an enzyme concentration of 2 micromolar and concentrations of 4S-TTP between 2.5 and 30 micromolar. The apparent pseudo-first-order rate constant approached a limiting value of 3-4 reciprocal seconds at high 4S-TTP concentration. This rate constant is about tenfold smaller than that reported for the pre-steady-state "burst" phase in the analogous reaction of TTP, which corresponds to the catalytic process within the TTP/enzyme/template-primer complex, and involves a conformational change of the complex followed by very fast covalent bond formation. The steady-state turnover rate constant of 1.8 reciprocal seconds for 4S-TTP is about 3-5 fold larger than that for TTP. These results suggest that substitution of sulfur for oxygen in the thymine base markedly decreases the rate of the one or both of the steps involved in catalysis, while slightly increasing the rate of dissociation of the oligonucleotide product. As a result, the rate of the catalytic process with 4S-TTP approaches that of dissociation of the oligonucleotide product, such that the step(s) in this process may be partially rate limiting for the overall reaction. In contrast, for elongation of this oligonucleotide primer by a single residue of the normal substrate, TTP, the catalytic process is fast and dissociation of the oligonucleotide product is rate limiting under steady-state conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31108-07 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adenosine Receptor Agonists and Antagonists

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	Y. Shin	IRTA	LBC, NIDDK
	Dan Shi	IRTA (Supplemental)	LBC, NIDDK
	W. Padgett	Biologist	LBC, NIDDK

COOPERATING UNITS *(if any)*

R. Olsson, U. So. Fla., Tampa, FL; C. Muller, U. Wurzberg, Germany; B. Fredholm, Karolinska Inst., Stockholm, Sweden.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adenosine regulates a wide range of physiological functions through interaction with at least two major classes of adenosine receptors. The A1 and A3 classes of adenosine receptors are inhibitory to adenylate cyclase, while the A2 class is stimulatory to adenylate cyclase. Subclasses of adenosine receptors also occur. Some of these are inhibitory to calcium channels, some are stimulatory to potassium channels, some activate guanylate cyclase, some modulate phospholipid turn-over and some cause smooth muscle relaxation. Further pyrazolopyridine were synthesized and analyzed for antagonist activity at A_1 and A_2 adenosine receptors and for effects on diazepam-binding to $GABA_A$ receptors. Most were nonselective or were A_1 receptor selective antagonists. Certain pyrazolopyridines were very potent in stimulating diazepam-binding and might therefore have anxiolytic activity. Others showed only inhibitory activity towards diazepam-binding. Adenoregulin, an amphiphilic peptide from a hylid frog stimulated binding of agonists to Gi-protein-coupled receptors apparently due to conversion of the receptor-G-protein complex to a high affinity guanylnucleotide free state. Congeneric peptides from the hylid frog inhibited binding of agonists and are under investigation. Chronic caffeine or theophylline ingestion altered levels of a variety of central receptors. Only β-adrenergic and nicotinic receptors appeared to be down-regulated, suggestive of xanthine-induced increases in norepinephrine and acetylcholine turnover. Chronic theobromine, a very weak adenosine receptor antagonist, and chronic isobutylmethylxanthine, a potent adenosine receptor antagonist and, in addition, a potent phosphodiesterase inhibitor, caused, like caffeine/theophylline, upregulation of A_1-adenosine receptors, but did not alter levels to other receptors. Behavioral responses to various receptor agonists/antagonists were altered after caffeine, consonant with observed changes in receptor levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31111-25 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stereopopulation Control in Drug Delivery and Enzyme Simulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	Michael M. King	Special Volunteer	GWU

COOPERATING UNITS *(if any)*

Y. Ueno, Nagoya, Japan; Y. Takeuchi, Toyama, Japan; W. Antkowiak, Poznan, Poland; J. Flippen-Anderson, NRL, Washington, DC; C. Takimoto, NCI; W. Dürckheimer, Frankfurt, FRG

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have synthesized a wide variety of intramolecular systems which simulate the enzyme-substrate complex by having the molecule frozen into a single conformation very favorable to bringing the interacting groups into the closest possible juxtaposition (stereopopulation control). These compounds undergo intramolecular reactions at rates approaching those catalyzed by enzymes (but independently of any functional group assistance). As part of our studies of practical applications of stereopopulation control, we have explored the use of various SPC-derivatives of drugs as pro-prodrugs. The intent is to facilitate transport from the gut to the circulatory system to the desired site of action by temporary masking of charge and polarity within the drug, by improvement in lipophilicity and by protection against enzymatic destruction. Recent studies have concentrated on o-nitrophenylpropionic acid derivatives as carriers. The nitro group is reduced enzymatically, and the resulting amine attacks an amide or ester bond intramolecularly to release the drug. Rapid attack by the amine is ensured by placing a gem-dimethyl group on the adjacent carbon atom. Efficient enzymatic reduction of the nitro group, however, requires a reducing, or hypoxic, environment. Thus, such a carrier is ideally suited to effect selective drug release at an hypoxic cancer cell, while remaining largely unaffected at the surface of a normal cell with high oxygen content. Incubation of our first carrier-taxol complex with a clone of breast cancer cells has shown levels of toxicity comparable to those of taxol itself. Studies with additional complexes are in progress, on a scale large enough to determine the fate of the carriers. Much larger amounts of carrier-drug complex are being prepared for whole animal studies, which are necessary in order to evaluate the degree of selective taxol release at the hypoxic cancer cell. Additional studies are in progress to link these carriers to other cancer chemotherapeutic agents, such as 5-fluorouracil.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31112-19 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Chemistry of Imidazoles and Bioimidazoles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Louis A. Cohen Chief, Section on Biochem. Mech. NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No activity during this period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31113-19 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Halogenated Biogenic Amines in Biochemistry and Pharmacology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Kenneth L. Kirk	Chief, Section on Drug-Receptor Interactions	LBC, NIDDK
Other:			
	Jun-ying Nie	IRTA Fellow	LBC, NIDDK
	Mona Singh	IRTA Fellow	LBC, NIDDK
	Milton Sloan	IRTA Fellow	LBC, NIDDK
	Jurgen Wess	Visiting Assoc.	LBC, NIDDK
	J. W. Daly	Chief	LBC, NIDDK

COOPERATING UNITS (if any)

M. Channing, D. Kiesewetter (CC, Dept. of Nuclear Medicine), D.S. Goldstein (HE, NHLBI), V. Pike (Hammersmith Hospital, London)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Drug-Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.6	3.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Biogenic amines play key roles in neurotransmission, metabolism, and in control of various physiological processes. Using a variety of synthetic methodologies, including novel procedures developed by us, we have prepared a series of biogenic amines with flourine substituted at various ring-positions. By virtue of its very small size and high electronegativity, flourine is a very favorable replacement for hydrogen in these analogues. The biological properties and usefulness of these ring-fluorinated biogenic amines have proved to be extremely rewarding and continue to find applications in a multitude of studies, including research on the mechanisms of transport, storage, release, metabolism, and modes of action of these amines. Of particular significance was the discovery that 6-flouronorepinephrine is a selective alpha-adrenergic agonist and 2-fluoronorepinephrine is a selective beta-adrenergic agonist. Mechanisms considered to explain these results include: 1) a direct effect of the C-F bond on agonist-receptor interaction or 2) an indirect effect of the C-F bond on the conformation of the ethanolamine side-chain. The results of testing of new analogues synthesized to probe these mechanisms have not clearly differentiated between these two basic mechanisms. Binding of fluorinated analogues to cloned wild-type and mutant adrenergic receptors has been carried out in an attempt to identify specific sites on the receptor protein responsible for flourine-induced adrenergic selectivities. We have developed stereoselective syntheses of threo-2- and 6-fluorodihydroxyphenylserine (fluoro-DOPS). A new synthesis of fluorinated and polyfluorinated veratraldehydes, based on direct electrophilic fluorination, has been realized, and we have prepared all mono- and difluorinated analogues of veratraldehyde as well as trifluorovertraldehyde. These are being used to prepare the corresponding biogenic amines and their amino acid precursors. Examination of the interactions of the mono-, di-, and trifluoroanalogues of dopamine with D-1, D-2, and D-3 dopamine receptors has been carried out. Aminocyclopropane carboxylic acid (ACCA) has been shouwn by others to provide protection from damage due to stroke of central neurons. Side chain protected fluoroaminocyclopropane carboxylic acids have

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 31114-13 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Significance of Ligand Tautomerism in Biorecognition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No activity during this period

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31115-12 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Functionalized Congeners of Bioactive Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. A. Jacobson	Research Chemist	LBC, NIDDK
Others:	Jeong, Hea OK	Visiting Fellow	LBC, NIDDK
	Ji, Xiao Duo	Visiting Associate	LBC, NIDDK
	Kim, Jeongho	Visiting Fellow	LBC, NIDDK
	Melman, Neli	Special Volunteer	LBC, NIDDK
	Yoburn, Josh	Summer IRTA	LBC, NIDDK
	Siddiqi, Suhaib M.	IRTA Fellow	LBC, NIDDK
	Van Rhee, Albert M.	Special Volunteer	LBC, NIDDK

COOPERATING UNITS (if any) A. Ijzerman (Univ. Leiden, The Netherlands); G. Stiles (Duke Univ.); Y. Karton (Israel Inst. Biol. Res., Nes Ziona, Israel); K. Harden (Univ. N. Carolina); G. Burnstock (U. College London)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Molecular Recognition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.6	4.3	0.3

CHECK APPROPRIATE BOXES:
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent work in our laboratory has demonstrated that certain drugs may be attached to well-defined "carrier" molecules and still retain the ability to bind to the receptor site and effect biological activity. This synthetic strategy for the attachment of drugs to carriers is termed the "functionalized congener" approach. The "carrier" molecule may be many times larger than the parent drug; indeed there is practically no maximum size limitation for a fully potent analog. Unlike the prodrug approach or the immobilization of drugs for slow release, the "functionalized congener" approach is designed to produce analogs for which no metabolic cleavage step is necessary for activation. Moreover, the attachment of the drug to a "carrier" such as a peptide may result in enhanced affinity at an extracellular receptor site and an improvement in the pharmacological profile of the parent drug.

Purine derivatives containing attached chains were developed as functionalized congeners for adenosine receptors and for ATP receptors. Reporter groups such as fluorescent dyes have been covalently attached resulting in receptor probes of relatively high affinity. Sites for chain derivatization on the structures of ATP and selective muscarinic antagonists have been located. A3 adenosine receptors are important in the regulation of CNS, cardiac, inflammatory; and reproductive functions. We have developed the only selective agetns for this novel receptor. Selective A3 agonists are effective in the treatment of neurodegenerative diseases. Antagonists have been proposed to have anti-inflammatory properties. New xanthine and adenosine derivatives are being synthesized, screened for A3-receptor selectivity, and later tested in vivo. Irreversibly-binding receptor probes are being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31116-08 LBC

PERIOD COVERED
October 1, 1994 to September 30,1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Prosthetic Groups for Labeling of Functionalized Drugs and Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. A. Jacobson	Research Chemist	LBC, NIDDK
Others:	Xiao-duo Ji	Visiting Associate	LBC, NIDDK

COOPERATING UNITS (if any)
G. Stiles (Duke University)

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Molecular Recognition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The use of radioisotopes to label organic compounds for use in diagnostic nuclear medicine is well documented in the literature. It has been found that certain radiolabeled compounds will localize in the brain, heart, or in other target organs or tissues to a sufficient level to allow for imaging thereof.

Binding sites for certain drugs in an animal or organ may be localized as a result of the synthesis of high specific activity radiolabeled analogs which have high affinity for that binding site. Prosthetic groups may be attached to a drug or other receptor ligand for the purpose of efficient and selective chemical capture of a particular radioisotope. Having developed functionalized congeners of theophylline and other drugs acting at adenosine receptors, we are now developing prosthetic groups for radioisotopes such as 18-F, 123-I, and 125-I, to be coupled to these functionalized drug molecules. The prosthetic groups contain amino or carboxylic groups which are to be condensed covalently to functionalized drugs to give conjugates of high affinity at a particular receptor, or drugs that bind the label irreversibly (trifunctional reagents).

A general approach for reversible affinity labeling of receptors has been developed. The objective is to carry out a series of chemical modifications resulting in a covalently-modified, yet functionally-regenerated receptor protein that also may contain a reporter group. The ligand recognition site of A1-adenosine receptors in bovine brain membranes was probed to demonstrate the feasibility of this approach. Use of disulfide or ester linkages, intended for cleavage by exposure of the labeled receptor to either reducing reagents or hydroxylamine, respectively, was considered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31117-08 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Drugs Acting at Adenosine Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. A. Jacobson	Research Chemist	LBC, NIDDK
Others:	M. Beenhakker	Special Volunteer	LBC, NIDDK
	Mellonie Boyd	SEEP Program	LBC, NIDDK
	Xiao Duo Ji	Visiting Associate	LBC, NIDDK
	Jeongho Kim	Visiting Fellow	LBC, NIDDK
	Neli Melman	Special Volunteer	LBC, NIDDK
	Dag von Lubitz	Guest Researcher	LBC, NIDDK

COOPERATING UNITS (if any) A. Shainberg (Bar Ilan Univ. Israel); G. Stiles (Duke Univ.); L. Heppel (Cornell Univ.); S. Deutsch (VA Hosp. D.C.); A.P. Ijzerman (Ctr. for Bio-Pharmaceut. Sci., Leiden, The Netherlands); P. Lin (Hahnemann Univ., Philadelphia PA)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Molecular Recognition

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The extracellular adenosine receptor has a modulatory role in the nervous, circulatory, endocrine and immunological systems. The prospect of harnessing these effects specifically for therapeutic purposes is attractive. Recently this project has focused on the effects of adenosine agonists and antagonists in the central nervous system and on the possiblity of therapeutics for treating neurodegenerative diseases. Chronic treatment with an adenosine agonist improves spaital memory retention and acquisition and indicates necessity of further studies directed towards Alzheimer's disease. Neuroprotective effects of acutely administered A1 receptor agonists and chronically administered A1 receptor antagonists have been demonstrated. Adenosine agonists prevent convulsions in several chemical and electrical seizure models and protect against excitotoxic neurodegeneration agonists and antagonists suggests some adaptation (sensitization by antagonists/desensitization by agonists) of the mechanism of receptor activation, either at the lefel of receptor or second messenger. An A3 agonist proved to be highly cerebroprotective in an ischemic model in gerbils. In summary, highly selective adenosine analogues may have therapeutic potential in treatment of cerebral ischemia/stroke and possibly other neurodegenerative disorders as well. Since the three major subtypes of adenosine receptors have been cloned it has been possible to conduct molecular modeling of the redeptor protein, based on sequence analyses and computerized energy minimizations. A hypothesis concerning the mode of binding to ligands to adenosine receptors has been derived. This hypothesis is consistent with pharmacological observations and site directed mutagenesis experiments, in which key histidyl residues have been replaced by other amino acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 31118-06 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bioindoles and Oxindoles as Medicinal and Diagnostic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	R. G. Rajeswaran	Visiting Fellow	NIDDK-LBC

COOPERATING UNITS *(if any)*
Peter Kador, NEI; David Klein, NICHD; Robert Phillips, Athens, GA; S. Gronowitz, Lund, Sweden; J. Vanecek, Prague, Czech Republic; D. Sugden, Oxford, UK; M. Katayama, Nagoya, Japan; J. Hadden, St. Petersburg, FL

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interest in the physiological activities and potential clinical uses of the pineal hormone melatonin (N-acetyl-5-methoxytryptamine) has grown enormously in recent years. The hormone has been implicated in the regulation of phenomena as diverse as the sleep-wake cycle, depression, sexual urge, neuroimmunomodulation, mammary cancer, etc. Such multiplicity of actions in different tissues suggests a multiplicity of receptors for the same molecule. Studies to date indicate a receptor pocket for a large nonpolar group at C-2, such as iodine or phenyl, these analogs of melatonin showing greatly increased binding capacity. Thus, a bulky, chemically reactive substituent at the same position should provide an affinity label for one or more classes of receptor. Stepwise chemical syntheses of such molecules would very involved; we have, therefore, aimed for direct synthesis by use of the carbonyl function of the corresponding oxindole. We have now developed reversible blocking groups for the oxindole nitrogen which render the ring carbonyl more ketonic in character and amenable to nucleophilic addition. The first test of this route has been successful, in that Grignard addition provides a series of 2-alkyl or 2-aryl indoles. Thus, a 3-step synthesis is now available to provide a variety of melatonin analogs with functional groups at C-2.

Melatonin has been claimed to show immunostimulant activity and even some positive effect against breast cancer, but the mechanism of its action remains unknown. A minor metabolic pathway for melatonin involves oxidative cleavage of the pyrrole ring to give melakynurenine, a product analogous to that formed by oxidative cleavage of tryptophan. Comparison of the geometry of this metabolite with that of muramic acid, the glucosamine component of muramyl dipeptide, shows a remarkable degree of resemblance. Since muramyl dipeptide is the most frequently used adjuvant in vaccine preparations, it is quite possible that the immunoadjuvant property of melatonin may actually arise from melakynurenine. We have prepared melakynurenine and several analogs thereof; potential immunostimulant activities will soon be evaluated by Prof. John Hadden, Univ. of South Florida School of Medicine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31119-06 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Novel Amino Acids for Conformational and Stereochemical Constraints in Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No activity this year

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31120-06 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fluorinated Analogues of Bioactive Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been temporarily discontinued

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 31121-05 LBC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Biology of Novel Pyrimidine and Purine Nucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No activity during this period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31122-05 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Medicinal Agents Based on Bioheteroaromtic Ring Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIDDK-LBC
Other:	Rahul Jain	Visiting Fellow	NIDDK-LBC
	Michael King	Special Volunteer	GWU

COOPERATING UNITS (if any)

J. Golenser, Jerusalem, Israel; Dept. of Parasitology, WRAIR, Washington, DC

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have found 2-iodo-L-histidine and 2-iodohistamine to be potent antimalarial agents against drug-resistant strains of Plasmodium falciparum. The compounds are effective in monkeys for only 48 hrs, and we have shown that inactivation may be the result of nonenzymatic deiodination by any sulfhydryl compound present in serum or tissue. The facts that 2-iodohistidine does not block protein synthesis in the parasite, and that the corresponding 2-bromo and 2-chloro compounds are inactive, led us to speculate that the 2-iodo compound operates by plugging one or more holes in the erythrocyte membrane and, thus, deprives the parasite of nonamino acid nutrients obtained by diffusion through such holes. We have, therefore, synthesized and tested a broad range of metabolically stable analogues of the bioimidazoles, in which the ring substituents are close to iodine in size. We have developed a very specific and general method for C-2 alkylation of imidazoles. In vitro activity appears to depend on the extent to which a substituent projects from the imidazole ring, with the optimum size slightly larger than that of iodine (e.g., isopropyl). Analysis of geometry suggests that 1-alkyl derivatives may also be effective. A regiospecific synthesis was developed for 1-alkylhistidines and histamines. As anticipated, the 1-isopropyl derivatives are almost as active as 2-isopropyl. Further analysis suggests that 4-iodo-2-isohistamine and 4-iodo-1-(aminoethyl)-imidazole, in which the iodo function will be metabolically stable, should also be effective. These compounds have been submitted for testing.

Drug resistance by the parasite and multidrug resistance in cancer chemotherapy both involve drug ejection by an ATP-driven G-protein pump; in both cases, resistance can be partially overcome by calcium channel blockers, suggesting that pore size is comparable for the two systems. Accordingly, a number of our active antimalarial agents have been submitted for testing to overcome multidrug resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 32001-04 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Multifunctional Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	B. Vithal Shetty	Guest Researcher	LBC, NIDDK
Others:			
	K. L. Kirk	Chief, Section on Drug-Receptor Interactions	LBC, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Drug-Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A novel approach to the design of new antimicrobial agents has been developed. The overall purpose of the program is the rational design and synthesis of compounds that have combined anti-bacterial, anti-viral and anti-fungal activity. Specifically targeted in the rational design are enveloped viruses such as herpes viruses and HIV. These compounds have novel sturctural features which include a dimeric attachment of a known hydrophobic antimetabolite (for example, aminoadamantane analogues) through a very hydrophilic bridge. These structural features combine to provide the potential for a novel mechanism for attachment of the toxic moiety to microbial organisms. Thus, the hydrophilic portion of the molecule has a high affinity for glycoprotein components of the microbial cell wall. This affinity will deliver the toxic moieties to the cell surface, providing a mechanism for efficient activity. For example, the potential exists for inhibition of replication of such viruses as HIV and, through the attachment of the toxic molecule to the viral coat, a mechanism for killing the virus.

Initial testing of several bisadamantamine analogues (U.S. Patent No. 5,221,693) revealed potent activity against gram positive and gram negative bacteria, fungi, yeast and enveloped viruses. Such combined activity potentially could be extremely useful in treatment of immunosuppressed patients. Two compounds were found to be very active against an amoeba called acanthamoeba polyphargia cyst. Current modifications to these prototypical molecules include introduction of a sulfoxide group in the bridging alkylene group to provide increased hydrophilic interactions. Also, aromatic rings have been introduced adjacent to the adamantane rings. These modifications have led to marked improvement in the level of anti-viral activity, and concomitant reduce cytotoxicity. For example, a bis-Schiff base prepared from 2-adamantanone and 4,4'-diaminophenylsulfone has been found to have potent activity against HIV infection in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 32002-03 LBC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis and Biochemistry of Ascorbic Acid Analogues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth L. Kirk	Chief, Section on Drug Receptor Interactions	LBC, NIDDK
Others:			
	Ping Ge	IRTA FELLOW	LBC, NIDDK

COOPERATING UNITS *(if any)*

Mark Levine, Richard Welch, Yaohui Wang, Steve Rumsey (Nutrition Program, LCBG, NIDDK)

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Drug-Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ascorbic acid (vitamin C), a dietary requirement for human health, is an electron donor for several enzymatic actions, functions as an antioxidant, and is implicated in host defense mechanisms, endocrine function and the visual process (lens). Recent renewed interest in the biochemistry of ascorbic acid has been prompted by the realization that relatively little is known concerning the concentrations of the vitamin required for optimum functioning of these several roles. In the case of enzymatic reactions, optimal rate of a process is defined as that concentration that allows the reaction to reach Vmax without toxicity. As part of a program to determine these concentrations, in situ kinetic measurements have been carried out for certain vitamin C-linked reactions. In addition to examination of functional roles of vitamin C, recent characterization of efficient transport mechanisms that translocate vitamin C across cellular membranes has emphasized the importance of the vitamin to biological processes. Kinetic parameters of these transport mechanisms are also being determined.

Kinetic measurements of transport inhibition of 6-deoxy-6-haloascorbic acid analogues have clearly demonstrated the presence of separate pathways for the translocation of ascorbic acid and dehydroascorbic acid. Using our route to 6-aryloxy-ascorbic acid analogues, we have prepared radiolabeled functionalized analogues having the potential for irreversible binding to the ascorbic acid transporter. To investigate the importance of the 2-hydroxyl group on ascorbic acid activity, we have prepared 2-deoxy-ascorbic acid. This analogue will be used as a starting material for the attempted preparation of the unknown 2-deoxy-2-halo ascorbic acids, including 2-deoxy-2-fluoro-ascorbic acid, an isosteric and isopolar, non-oxidizable, analogue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 32003-03 LBC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Neurotransmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Jürgen Wess, Ph.D.	Visiting Scientist	NIDDK-LBC
Others:	Torsten Schöneberg, Ph.D.	Special Volunteer	NIDDK-LBC
	Nathalie Blin, Ph.D.	Visiting Fellow	NIDDK-LBC
	Jie Liu, Ph.D.	IRTA Fellow	NIDDK-LBC
	June Yun, Ms.	Pre-IRTA Fellow	NIDDK-LBC

COOPERATING UNITS (if any)

C. Felder, Ph.D., NIMH, LCB; B. Conklin, Ph.D., University of California, San Francisco, California

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Drug Receptor Interactions

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To study how G protein-coupled receptors (GPCRs) are assembled and function at a molecular level, various members of the muscarinic acetylcholine receptor family (m1-m5) were used as model systems.

Coexpression studies with m3 muscarinic receptor fragments (obtained by splitting the receptor in all three intracellular (i1-i3) and all three extracellular loops) indicated that GPCRs are assembled from multiple autonomous folding domains. This finding was also confirmed by immunocytochemical studies showing that most receptor fragments examined were stably incorporated into lipid bilayers (including the plasma membrane), even when expressed alone.

Hybrid m2/m5 muscarinic receptors that contain m5 sequence in transmembrane domain I (TM I) and m2 sequence in TM VII are known to be pharmacologically inactive. We could demonstrate that such misfolded hybrid receptors can be pharmacologically rescued by introduction of a single point mutation into either TM I (*m5*Thr37->*m2*Ala30) or TM VII (*m2*Thr423->*m5*His478). This finding allows predictions as to how TM I and TM VII are oriented relative to each other.

To identify single amino acids required for selective coupling of the m3 receptor to G proteins of the Gq/11 family, distinct intracellular segments/amino acids of the m3 receptor were systematically substituted into the Gi/o-coupled m2 receptor. Functional analysis of the resulting mutant receptors showed that selective recognition of Gq/11 by the m3 receptor is determined by a limited number of amino acids located in the i2 loop and at the N- and C-terminus of the i3 domain.

To delineate specific sites of contact between the m2 receptor and Gi/o proteins, coexpression studies were carried out with mutant m2 receptors and G protein αq-subunits in which the C-terminal portion was replaced with sequences derived from αi2 or αo. These studies showed that a short region in the m2 receptor, located at the junction between the i3 loop and TM VI, can recognize the C-terminus of Gαi (Gαo) subunits with high selectivity.

Since all GPCRs share a similar molecular architecture, our findings should be of broad general relevance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33000-29 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Functions Involved in Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Martin Gellert, Ph.D., Chief, Section on Molecular Genetics, LMB/NIDDK

OTHERS: Sergei Kozyavkin, Ph.D., Visiting Associate, LMB/NIDDK
Regis Krah, Ph.D., IRTA Fellow, LMB/NIDDK
Mary H. O'Dea, Research Chemist, LMB/NIDDK

COOPERATING UNITS *(if any)*

Dr. M. Couturier, U. of Brussels, Belgium (foreign)
Dr. A.D. Bates, U. of Liverpool, U.K. (foreign)

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reverse DNA gyrases of thermophilic bacteria are enzymes that introduce positive supercoiling into circular DNA at the expense of ATP hydrolysis. We previously described an enzyme of this class with an unusual two-subunit structure in the hyperthermophile *Methanopyrus kandleri*. The larger subunit (MW 138,000) has the ATPase activity while the smaller (MW 41,000) carries out the DNA breakage-reunion step. This functional partition is similar to that of the eubacterial DNA gyrases.

We have now cloned and sequenced the genes for both subunits. Remarkably, the characteristic domain structure found in a single protein chain in all other type I topoisomerases is divided between the two subunits in this enzyme.

Another topoisomerase from the same bacterium, topoisomerase V, has been shown to retain activity at temperatures as high as 122° C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 33001-11 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Immunoglobulin Gene Rearrangement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Martin Gellert, Ph.D., Chief, Section on Molecular Genetics, LMB/NIDDK

OTHERS: Joanne Hesse, Ph.D., Research Chemist, LMB/NIDDK
Fraser McBlane, Ph.D., Visiting Associate, LMB/NIDDK
Moshe Sadofsky, M.D., Ph.D. Sr. Staff Fellow, LMB/NIDDK
Dik Van Gent, Ph.D., Special Volunteer, LMB/NIDDK
Dale Ramsden, Ph.D., Special Volunteer, LMB/NIDDK

COOPERATING UNITS *(if any)*

Dr. M.J. Bosma, Fox Chase Cancer Center
Dr. M.A. Oettinger, Harvard Medical School

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.8	5.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The assembly of functional antigen receptor genes in lymphoid cells (generally known as V(D)J recombination) is known to depend on the products of the RAG-1 and RAG-2 genes, but it has not been clear whether these proteins act directly in recombination or whether they are indirect activators. Recombination is known to begin with a double-strand break in DNA, exactly at the border between each coding segment and its neighboring recombination signal sequence. We have now succeeded in reproducing this specific breakage step in a cell-free reaction requiring only the purified RAG-1 and RAG-2 proteins. Thus RAG-1 and RAG-2 act directly in V(D)J recombination, and contain all the information for the site specificity of the reaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33006-17 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Mechanism of Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kiyoshi Mizuuchi	Chief, Section on Genetic Mechanisms	NIDDK/LMB
Others:	Michiyo Mizuuchi	Visiting Associate	NIDDK/LMB
	Harri Savilahti	Visiting Fellow	NIDDK/LMB

COOPERATING UNITS *(if any)*

M. Clore, A. Gronenborn, J. Omichinski, R. Clubb - NIDDK/LCP
H. Nash, S.W. Yang - NIMH/LMB

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Genetic Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objective of this project is to uncover the molecular mechanisms of a variety of genetic rearrangements. The transposition reaction of bacteriophage Mu is studied as a model system. Critical steps in Mu transposition are a pair of DNA cleavages and strand transfers which generate a branched DNA intermediate. Efficient formation of this intermediate requires, besides the MuA protein which is the transposase, the following accessory factors: the MuB protein, the E. coli-encoded HU and IHF proteins, ATP, and Mg^{++}. MuA interacts with two different specific DNA sequences, one at the ends of the Mu genome and the other at the Mu operator. Interactions involving multiple MuA molecules, accessory protein factors and sequence elements on the donor DNA lead to formation of a stable protein-DNA complex in which the two Mu ends are synapsed by a tetramer of MuA. Next, a pair of single strand cuts are made to expose the 3′ ends of the Mu DNA. The cleaved donor DNA remains tightly associated with the MuA tetramer and this complex efficiently captures a 'target' DNA molecule provided it is bound by MuB. A staggered cut is introduced into the target DNA and the two 5′ ends are joined to the 3′ ends of the Mu DNA in a concerted reaction. The assembly process and the functional organization of the MuA tetramer-Mu DNA complex have been studied by making use of a variety of mutant MuA proteins with missing functional domains. Structurally and functionally important protein-DNA interactions within the stable complexes were analyzed by assembling the complexes from short Mu end DNA fragments and MuA under permissive reaction conditions, bypassing the need for many of the cofactors normally required for the process. The structure of the catalytic core domain of MuA has been determined by X-ray crystallography. The MuA catalytic subdomain shares remarkably similar structural arrangements with the core domain of HIV integrase which carries out similar biochemical reactions. In collaboration with scientists in LCP/NIDDK, the N-terminal domain of MuA was shown to have the 'winged helix-turn-helix' type of DNA binding structure by NMR

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34001-30 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chromatin Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gary Felsenfeld, Chief, Section on Physical Chemistry	LMB/NIDDK

OTHERS:	David Clark, Visiting Assoc.	LMB/NIDDK	Adam Bell, IRTA	LMB/NIDDK
	Dan Chen, IRTA	LMB/NIDDK	Pascale Nony, V. Fellow	LMB/NIDDK
	Cecelia Trainor, Staff Fellow	LMB/NIDDK	Henryk Eisenberg, V. Scien	LMB/NIDDK
	Rodolfo Ghirlando, V. Scien.	LMB/NIDDK	Vasily Studitsky, V. Assoc.	LMB/NIDDK
	Joan Boyes, V. Scien.	LMB/NIDDK	Michael Pikaart, IRTA	LMB/NIDDK

COOPERATING UNITS *(if any)*

LCP, NIDDK (J. Omichinski, G.M. Clore, A. Gronenborn); Dept. of Biophysics, King's College London (H. Gould, R. Hannon); NICHD, NIH (Heiner Westphal); University of Pittsburgh (T. Evans)

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
9.5	9.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of chromatin structure in the neighborhood of expressed genes. The globin gene family in chicken erythroid cells serves as a model system in which it is possible to study the mechanisms associated with regulation of the individual members of the family during erythroid development. We have continued our studies of locus control regions (LCRs) by investigating the generation of hypersensitive sites in the chicken beta globin locus control element. We show that generation of such a site is an all-or-none process, so that partially mutating the site reduces the fraction of sites that is active, rather than reducing the activity of every site. We have also extended studies of the insulator element at the 5' end of the chicken beta globin locus by showing that activity is distributed throughout the element, but more is contained in a small portion which functions well as an insulator when multiple copies are used. We have extended our studies of the interaction of the erythroid regulatory factor GATA-1 with its target DNA by showing that certain sequences have an unusually high affinity for this factor, suggesting an additional regulatory role. We have also continued studies of the way in which RNA polymerase transcribes through chromatin templates. Using a model system, we have shown that the polymerase is forced to pause in advancing on such templates because of internal DNA loop formation.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34002-30 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzyme Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David R. Davies	Section Chief, Molecular Structure	NIDDK/LMB
Others:	Mi Li	Visiting Associate	NIDDK/LMB
	Der-Ing Liao	IRTA	NIDDK/LMB
	Sangkee Rhee	Visiting Fellow	NIDDK/LMB
	Enid Silverton	Research Chemist	NIDDK/LMB

COOPERATING UNITS *(if any)*

Edith Miles, NIDDK   Alan Peterkovsky, NHLBI   Ira Pastan, NCI

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued work on the high resolution structure of the bifunctional enzyme complex tryptophan synthase. We have examined mutants in order to understand the mechanism of action of both enzymes and the mechanism of communication between the two active sites. The native enzyme structure has been refined to 1.9A resolution. A sodium binding site has been confirmed by the examination of enzyme in the presence of K+ and Cs+ ions. Changing the cation produced unexpected changes in the structure that can be related to the allosteric properties of the enzyme and the channeling of the indole intermediate between the active sites.

Domain III of pseudomonas Aeruginosa exotoxin has been crystallized and the structure determined. The enzyme was crystallized with NAD which undergoes slow hydrolysis to give AMP and nicotinamide. We have now been able to bind a non-hydrolysable analog of NAD that permits us to see the NAD binding site.

The 30kD N-terminal domain of enzyme 1 of (PTS), the phosphoenolpyruvate: sugarphosphotransferase system has been crystallized and the three-dimensional structure determined.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34003-27 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytokines, Receptors and Immune System Proteins: DNA Structures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David R. Davies	Section Chief, Molecular Structure	NIDDK/LMB
Others:	Susan Chacko	IRTA	NIDDK/LMB
	Enid Silverton	Research Chemist	NIDDK/LMB
	Gerson H. Cohen	Research Chemist	NIDDK/LMB
	Der-Ing Liao	Visiting Associate	NIDDK/LMB

COOPERATING UNITS *(if any)*

Peter Sun, NIAID   Warren Leonard, NHLBI   H. Todd Miles, NIDDK/LMB

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The complexes of HyHEL-5 Fab with mutant lysozymes involving a conservative amino acid change have been crystallized, and the structures determined.

Extensive crystallization investigations with interleukin II receptors have yielded very small crystals of complexes with the alpha receptor.

Many data sets have been collected on a triplex DNA construct.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 35000-31 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical and Structural Investigations of Nucleic Acids and Related Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Todd Miles, Ph.D.	Chief, Section on Organic Chemistry	LMB/NIDDK
OTHERS:	F.B. Howard, Ph.D.	Research Chemist	LMB/NIDDK
	J. Frazier	Research Chemist	LMB/NIDDK
	Keliang Liu, Ph.D.	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*

David R. Davies, LMB/NIDDK
Philip Ross LMB/NIDDK
R. Raghunathan, NCI/NIH
Girjesh Govil, TIFR, Bombay, India

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Organic Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new homopolynucleotide triple helix poly dT. 2poly dA has been discovered and characterized. Detected by CD mixing curves, the complex is the product of an exothermic reaction of dA.dT with dA at moderate temperature and high salt concentration. At intermediate temperature the double helix undergoes a novel double disproportionation reaction to form both the new triple helix and the familiar dA.2dT. Differential scanning calorimetry was used to characterize the system thermodynamically. Complete phase diagrams were determined in NaCl, NaBr, and $NaClO_4$.

We had previously obtained the first crystals of DNA triple helices and observed fiber-type X-ray diffraction rather than the expected crystal diffraction. We have refined the molecular modeling to obtain good agreement with Fourier transforms calculated from the X-ray data, providing experimental support for the model. Helical parameters and coordinates were obtained for two distinct triple helices.

A search of numerous sequences to find a triple helix suitable for a crystallographic structure determination produced only crystals that diffracted like fibers, suggesting that this behavior is an intrinsic property of triplexes, We have therefore investigated mixed structures and have now obtained normal crystal-type X-ray diffraction from DNA sequences containing both triple and double helical portions. Diffraction is observed to 2.8 A. Dr. Davies and members of his group have begun efforts to solve the structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36003-11 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nonheritable Antibiotic Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. L. Rosner, Ph.D., Research Biologist, LMB/NIDDK

Others: P.M.R. Achary, Ph.D., Guest Researcher, LMB/NIDDK

COOPERATING UNITS *(if any)*

R. G. Martin, M.D., Medical Researcher, LMB/NIDDK
R.E. Wolfe, Jr., UMBC, Catonsville, MD

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. The emergence of multiply antibiotic resistant bacteria threatens the efficacy of antibiotics in combating infectious diseases. Among diverse microbial antibiotic resistance mechanisms, the mar regulon of Escherichia coli is unique in that it is inducible by agents used in clinical situations: the antibiotics chloramphenicol and tetracycline and aromatic weak acids such as salicylate and acetylsalicylate. These agents counter the marR-encoded repression of the marRAB operon; the induced MarA product then activates transcription of a dozen diverse genes (the mar regulon) leading to multiple antibiotic and superoxide resistances. We have now provided a mechanistic basis for these actions by purifying MarR and MarA proteins and studying their interaction with DNA in vitro. MarR was found to bind the mar promoter at 2 sets of sequences, 1 of which is likely to prevent mar transcription. Salicylate bound to MarR and decreased its affinity for the mar promoter DNA. MarA enhanced the transcription of various mar regulon promoters in a purified in vitro transcription system. MarA also bound specifically to a 'marbox' sequence in the mar promoter region. Deletions of this and adjacent sequences affected mar promoter activity in vitro and in vivo. Thus, MarR and MarA regulate the operon both negatively and positively, perhaps to enable rapid shut-down and start-up of the system.

B. We previously described the susceptibility of peroxidase mutants of E. coli and Salmonella typhimurium to isoniazid (INH), a clinically important antituberculosis drug. We now find that INH induces the SOS response: induction of lambda prophage, cellular filamentation, sulA expression, and mutation in the Ames Test. Since SOS is induced by agents that make single-stranded breaks in DNA, this implies that INH can damage DNA. It remains to be seen whether INH is mutagenic for Mycobacterium tuberculosis and whether it enhances the emergence of antibiotic resistant mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 36104-14 LMB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thermal Measurements of Biomolecular Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip D. Ross, Ph.D.	Research Chemist	LMB/NIDDK
OTHERS:	Frank B. Howard	Research Chemist	LMB/NIDDK
	Mikhail V. Rekharsky	Research Chemist	LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology, DIR/NIDDK

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studying the thermodynamics of protein-DNA association, we have reported the effects of a base-pair substitution and mutation of an amino acid ($Glu_{27} \rightarrow Val_{27}$) at a single site in the Cro-DNA complex (Proc. Natl. Acad. Sci USA, 8180, 1992). This work raised the question of whether these perturbation arose from interactions of the reactants with the solvent or from interactions within the protein-DNA complex or some combination thereof. In order to address these questions we have carried out model system studies of small molecule binding to α- and β-cyclodextrins which are hollow truncated conical substrates with a moderately hydrophobic core. This receptor exerts stringent size requirements upon the bound molecule, at the level of the radius of a single atom. The most important result of these studies is that the changes in the thermodynamic parameters are associated with the portions of the ligand molecule that undergo an alteration in its environment, thus the observed perturbations are at a sub-molecular level. We have obtained complete thermodynamic profiles for several examples of a single hydrogen-bond and a single hydrophobic interaction. For a closely related series of compounds, differences in interactions with the solvent are minimal, therefore indicating that the thermodynamic effects we have observed in our protein-DNA studies reflect differing interactions within the protein-DNA complex. Having gained this quantitative prerequisite knowledge from these model system studies, we are now in a position to carry out interpretable further studies altering the remaining base-pairs of the operator DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36105-13 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influences of Macromolecular Crowding on Biochemical Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. B. Zimmerman, Ph.D.	Research Chemist	LMB/NIDDK
OTHERS:	L. D. Murphy	Research Biologist	LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The DNA of the bacterial chromosome is localized in a compact body called the nucleoid. Bacterial nucleoids have been extensively studied, but the basis of nucleoid stabilization has remained elusive. The nucleoid is immersed in a cytoplasm which is very concentrated in macromolecules, giving a potential for large macromolecular crowding effects. The following results suggest that crowding-enhancement of the interaction between DNA-binding proteins and the cellular DNA may provide an important stabilizing force for the compact form of DNA in the bacterial nucleoid. These results include the first use of concentrated cell extracts to supply a crowded environment in model studies of the mechanisms of DNA compaction within bacterial or other cells.

DNA added to concentrated extracts of Escherichia coli undergoes a reversible transition to a readily-sedimentable ("condensed") form. The extract plays two roles in this transition, supplying both DNA-binding protein(s) and a crowded environment that increases protein binding and favors compact DNA conformations. The two roles are based on properties of fractions prepared by absorption of extracts with DNA-cellulose, and are consistent with model studies of condensation by combinations of purified DNA-binding materials (protein HU or spermidine) and concentrated solutions of crowding agents (albumin or PEG 8000); in each case, crowding agents and DNA-binding materials jointly reduced the amounts of each other required for condensation.

Condensation of the added DNA molecules caused large increases in the rate of cohesion between their complementary single-stranded termini. Cohesion products of lambda DNA made in vitro are a mixture of linear and circular aggregates, whereas those made in vivo are cyclic monomers. We suggest a simple mechanism based upon condensation at the site of viral injection which may explain this discrepancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36108-08 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Mechanism of Retroviral DNA Integration

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert Craigie, Ph.D., Visiting Scientist, LMB/NIDDK

Others: Alan Engelman, Ph.D., IRTA, LMB/NIDDK
Myung Soo Lee, Ph.D., IRTA, LMB/NIDDK
Tim Jenkins, Ph.D., Visiting Associate, LMB/NIDDK
Ronglan Zheng, Ph.D., IRTA, LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Genetic Mechanisms

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objective of this project is to uncover the molecular mechanisms of a variety of genetic rearrangements. The transposition reaction of bacteriophage Mu is studied as a model system. Critical steps in Mu transposition are a pair of DNA cleavages and strand transfers which generate a branched DNA intermediate. Efficient formation of this intermediate requires, besides the MuA protein which is the transposase, the following accessory factors: the MuB protein, the E. coli-encoded HU and IHF proteins, ATP, and Mg^{++}. MuA interacts with two different specific DNA sequences, one at the ends of the Mu genome and the other at the Mu operator. Interactions involving multiple MuA molecules, accessory protein factors and sequence elements on the donor DNA lead to formation of a stable protein-DNA complex in which the two Mu ends are synapsed by a tetramer of MuA. Next, a pair of single strand cuts are made to expose the 3' ends of the Mu DNA. The cleaved donor DNA remains tightly associated with the MuA tetramer and this complex efficiently captures a 'target' DNA molecule provided it is bound by MuB. A staggered cut is introduced into the target DNA and the two 5' ends are joined to the 3' ends of the Mu DNA in a concerted reaction. The assembly process and the functional organization of the MuA tetramer-Mu DNA complex have been studied by making use of a variety of mutant MuA proteins with missing functional domains. Structurally and functionally important protein-DNA interactions within the stable complexes were analyzed by assembling the complexes from short Mu end DNA fragments and MuA under permissive reaction conditions, bypassing the need for many of the cofactors normally required for the process. The structure of the catalytic core domain of MuA has been determined by X-ray crystallography. The Mua catalytic subdomain shares remarkably similar structural arrangements with the core domain of HIV integrase which carried out similar biochemical reactions. In collaboration with scientists in LCP/NIDDK, the N-terminal domain of MuA was shown to have the 'winged helix-turn-helix' type of DNA binding structure by NMR techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36109-08 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

AIDS Related Proteins: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David R. Davies	Section Chief, Molecular Structure	NIDDK/LMB
Others:	Alison B. Hickman	IRTA	NIDDK/LMB
	Frederick Dyda	IRTA	NIDDK/LMB

COOPERATING UNITS *(if any)*

Robert Craigie, NIDDK/LMB   Tim Jenkins, NIDDK/LMB   Alan Engelman, NIDDK/LMB

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Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The core domain of a soluble mutant of the HIV-1 integration protein has been crystallized and the crystal structure determined. The structure provides a platform for the design of inhibitors with antiviral properties. The enzyme has been shown to belong to a new superfamily of polynucleotidyl transferases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36114-05 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Studies of Molecular Recognition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eduardo A. Padlan	Visiting Scientist	LMB/NIDDK
Others:	Jennifer P. Tipper	Research Chemist	LMB/NIDDK
	Chantal Abergel	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) A molecular replacement solution of the crystal structure of the Fab of CC49, a murine monoclonal antibody against solid adenocarcinoma, has been refined at 3.5-Angstrom resolution.
2) A molecular replacement solution of the crystal structure of the Fab of TR1.9, a human autoantibody that binds to thyroid peroxidase, has been obtained and is being refined at 2.2-Angstrom resolution.
3) Crystals of a murine T-cell receptor alpha-chain variable domain have been obtained, X-ray data have been collected to 2.8-Angstrom resolution, and the determination of the structure by molecular replacement is in progress.
4) Crystallization of several proteins, including tyrosine hydroxylase, the extracellular domain of the alpha-chain and the intracellular domain of the gamma-chain of the human high-affinity receptor for IgE, and the Fab of the galactan-binding murine antibody X24, is being attempted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36115-05 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Potential Use of Catalytic Antibodies Against AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Eduardo A. Padlan Visiting Scientist LMB/NIDDK

COOPERATING UNITS *(if any)*

Ettore Appella, M.D., Medical Officer, LCB/NCI
Birgit A. Helm, Ph.D., Lecturer, Univ. of Sheffield, UK

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Peptidic transition-state analogs, corresponding to segments in the gp120 sequence, have been synthesized and used as immunogens for the production of murine monoclonal antibodies. Antibodies harvested from two of these clones produce peptide fragments when incubated with gp120/160, suggestive of proteolytic activity. The putative proteolytic products are being characterized by mass spectrometry. The clones are being grown to produce sufficient amounts of antibody to test for their ability to interfere with the binding of HIV-1 to helper T-cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36116-04 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Basis of Antibiotic Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert G. Martin, M.D. Medical Officer LMB/NIDDK

COOPERATING UNITS *(if any)*

Judah L. Rosner, LMB/NIDDK
R.E. Wolf, Jr., UMBC, Catonsville, MD

LAB/BRANCH

Laboratory Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the most serious medical problem facing the world is the diminishing efficacy of our armamentarium of antimicrobial agents. One of the ways in which enteric bacteria such as E. coli can become resistant to a broad spectrum of antibiotics is through induction of the multiple antibiotic resistance (mar) locus.

We have begun to dissect the regulation of the mar operon by purification of its two principle genetic products, MarR and MarA. MarR is a 16,000 dalton protein that binds to the promoter region in dimeric form at two sites: Site I lies between the -35 and -10 transcription initiation signals and is sufficient for repression of mar transcription by MarR. The second site, which contains the ribosome binding site and is located between the -10 signal and the first codon of MarR, is not essential for repression of transcription. MarR can bind salicylate but not antibiotics. MarR-promoter DNA complexes are disrupted when MarR binds to salicylate.

We have also obtained pure MarA, a protein of 15,500 that binds to, and stimulates the transcription of a dozen promoters dispersed throughout the E. coli chromosome. MarA induces bending of the DNA of these promoters. Some of the promoters require native RNA polymerase subunit, α; others can tolerate deletion of the carboxy-terminal domain of this subunit. Remarkably, we find that MarA also stimulates its own promoter. The binding site of MarA has been defined by footprint analysis and deletion mapping. The deletions have been analyzed both in vitro and in vivo for their promoter activity and for their ability to be stimulated by MarA and the MarA-related transactivator, SoxS. Although MarR and MarA bind to different sites, there is competition between their binding to promoter DNA.

Goal - To define in molecular terms how the mar operon like other molecular triage systems such as heat shock and s.o.s. repair alert the bacterium to the appearance of a noxious agent in the environment and stimulate the appropriate response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36117-02 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Gene Expression in *C. elegans*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Krause, Ph.D.	Senior Staff Fellow	LMB/NIDDK
OTHERS:	Morgan Park, Ph.D.	IRTA Fellow	LMB/NIDDK
	Robert Littlejohn	Biol. Lab. Technician	LMB/NIDDK

COOPERATING UNITS *(if any)*

Department of Embryology, Carnegie Institution of Washington (A. Fire, L. Chen)

LAB/BRANCH

Laboratory of Molecular Biology, DIR/NIDDK

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in the mechanisms controlling embryonic gene expression that result in the differentiation of omnipotent cells into one of several cell fates. Our model system revolves around myogenesis during embryonic development in the nematode *C. elegans*. Specifically, we have focused on the nematode homologs of genes encoding myogenic regulatory factors known to be critical in vertebrate myogenesis (eg. MyoD, E12/Da, and MEF2). We want to know what role, if any, these homologs play during nematode myogenesis. The simplicity of the nematode, both in terms of the anatomy and the developmental complexity, allows us to study the expression of these factors in detail. We can also address the function of these genes by inactivating them through mutations. We have shown that, as in mammals, CeMyoD is required to generate functional muscle during embryogenesis. However, unlike mammals in which the MyoD gene family is required for striated muscle determination, *C. elegans* striated muscle is determined and undergoes substantial differentiation in the absence of CeMyoD. The nematode homologs of both E12/Da and MEF2 also show differences in expression patterns and functions when compared to the vertebrates and *Drosophila*. Despite the extreme conservation of these factors, from nematodes to humans, the emerging picture is that the developmental processes in which they function are far less conserved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36118-02 LMB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analysis of Signal Transducing Proteins and Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James H. Hurley	Senior Staff Fellow	LMB/NIDDK
Others:	Gongyi Zhang	Visiting Fellow	LMB/NIDDK
	Jay Grobler	IRTA	LMB/NIDDK

COOPERATING UNITS *(if any)*

Antony M. Dean, Department of Biological Chemistry, The Chicago Medical School, North Chicago, IL   Peter M. Blumberg, LCCTP-NCI

LAB/BRANCH

Laboratory of Molecular Biology/NIDDK

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Protein kinase Cs (PKC) are a ubiquitous family of regulatory enzymes that associate with membranes and are activated by diacylglycerol or tumor promoting agonists such as phorbol esters. These lipophilic activators bind to conserved cysteine-rich domains. We have determined the crystal structure of the second cysteine-rich activator binding domain, known as 'cys2', of PKC delta by multiwavelength anomalous dispersion from native zinc ions. We have also determined the structure of the cys2 domain complex with the tumor promoting PKC activator phorbol ester. The structure explains the roles of phorbol esters and the cysteine-rich domain in the activation and membrane insertion of PKC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43006-19
MD

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Hyperparathyroidism: Etiology, Diagnosis and Treatment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.J. Marx, M.D.	Chief, Genet. & Endocrinol. Sec.	MDB, NIDDK
Others:		
A. Spiegel, M.D.	Chief,	MDB, NIDDK
M. Kester	Biologist	MDB, NIDDK

COOPERATING UNITS (if any)
Radiology Department, CC: (J. Doppman, T. Shawker)
Sugery Branch, NCI: (DL Fraker, R. Alexander)
Department of Endocrinology, Univ. of Florence, Italy (ML Brandi)

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)
☐(a) Human ☐(b) Human ☐(c) Neither
☐(a1) Minors
☐(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent or recurrent hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house clinical studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, ultrasonography, (reoperative and intra operative) radiothallium scanning, magnetic resonance imaging, CAT scanning, selective arteriograpy and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction. In vitro evaluation of parathyroid and other endocrine tissue involves tissue culture, chemistry and determination of linkage with DNA or RNA probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43008-13 MDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vitamin D Resistance and Related Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	S.J. Marx, M.D.	Chief, Genetics and Endocriol. Sec.	MDB, NIDDK
Others:	J. Barsony, M.D.	Visiting Scientist	MDB, NIDDK
	W. McKoy	Chemist	MDB, NIDDK
	M. Liberman, Ph.D.	Visiting Fellow	MDB, NIDDK

COOPERATING UNITS (if any)

Metabolism Unit, Beilinson Hospital, Betah Tiva, Israel (U. Liberman)
Hormone Action and Oncogenesis Section, NCI (G. Hager)

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Z01 DK 60050-01 LCBB.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43009-09

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Mineral Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Marx, M.D.	Chief, Genetics & Endocrinol. Sec.	MDB, NIDDK
Others:	W. McKoy	Chemist	MDB, NIDDK
	A. Spiegel, M.D.	Chief,	MDB, NIDDK
	M. Skarulis, M.D.	Senior Staff Fellow	ODIR, NIDDK
	D. Fraker, M.D.		Surg., NCI
	R. Alexander, M.D.		Surg., NCI
	L. Burns	Biologist	MDB, NIDDK
	M.B. Kester	Biologist	MDB, NIDDK

COOPERATING UNITS *(if any)*
Bevedere Medical Center - Carisle, PA (J.Green), Genetics Department - Yale University (A. Bale) Dept. of Endo. - Univ. of Florence Italy (ML Brandi) - Brigham Hosp. & Harvard Med (E.M. Brown).

LAB/BRANCH
Metabolic Disease Branch

SECTION
Genetics and Endocrinology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Disorders of mineral metabolism have been evaluated with methods extending from epidemiology to cellular and molecular biology. Two forms of familial hyperparathyroidism have been characterized in detail. Familial hypocalciuric hypercalcemia is an autosomal dominant trait associated with abnormal interactions with calcium in parathyroid and kidney. The affected gene in most kindreds encodes a G-protein linked calcium sensor, and missense mutations have been identified in many kindreds. Familial multiple endocrine neoplasia type 1 (FMEN1) is an autosomal dominant trait causing hyperfunction of parathyroids, pancreatic islet and anterior pituitary. It is associated with gradual but abnormal proliferation of the tissues affected. Plasma from affected persons shows high mitogenic activity upon cultured bovine parathyroid cells. This mitogenic activity in plasma may contribute to primary hyperparathyroidism in FMEN1. Analysis of blood and parathyroid tumor DNA has revealed that FMEN1 parathyroids often show clonal loss of alleles in the region of the FMEN1 gene on chromosome 11. Thus the FMEN1 gene probably functions as a tumor suppressor gene, analogous to the retinoblastoma gene. Analysis of sporadic parathyroid adenomas revealed that 25% showed allelic loss in a similar region. Thus the clonal inactivation of the FMEN1 gene may also be a contributing factor in many sporadic parathyroid adenomas. Tumors with allelic loss along chromosome 11 are being used to help delineate the minimal region of allelic loss which must harbor the men1 candidate gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK 43010-02

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Membrane targeting of G proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. Jones, M.D.	Senior Clinical Investigator	MDB, NIDDK
Others:	M. Degtyarev	Visiting Associate	MDB, NIDDK

COOPERATING UNITS (if any)
L. Jaffe, Univ. of Conn. Health Science center, Farmington, CT
P. Backlund, LGCB, NIMH

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH,Bethesda, MD

TOTAL STAFF YEARS 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Heterotrimeric G proteins couple cell surface receptors with intracellular effectors at the cytoplasmic face of membranes. The heterotrimer consists of a subunits which bind the guanine nucleotide and bg subunits which are tightly bound together. The membrane localization of G proteins is critical for their ability to relay signal between membrane-bound receptors and effectors. The a subunits undergo the post-translational modifications of myristoylation and palmitoylation. Myristoylation, the addition of a 14 carbon fatty acid to the amino-terminal glycine, is critical for the membrane localization of a subset of a subunits primarily because it increases the affinity of the a subunit for the bg complex. Palmitoylation is the reversible addition of the 16 carbon palmitic acid to cysteine residues through a thioester bond. Using a HPLC method, we found that approximately 75% of the subunits are modified with palmitate. Mutation of the cysteine at residue 3 in ai1 prevents palmitoylation. Palmitoylation like myristoylation is critical for the membrane attachment of ai1 but is not necessary for its formation of a heterotrimer with bg. The ai1 subunit undergoes palmitoylation when membrane localized and does not require myristoylation though myristoylation can slow the turnover of palmitoylation on ai1. Mutation of cysteine 11 in a12 prevents its palmitoylation but does not change its membrane localization. The loss of palmitoylation in a constitutively active mutant of a12 prevents the oncogenic transformation seen in NIH 3T3 cells after stable expression of the proteins. However, palmitoylation is not specifically required because myristoylation can substitute for palmitoylation and restore the transformed phenotype. Tunicamycin and nitric oxide (NO) were evaluated as possible modulators of palmitoylation. Tunicamycin can inhibit the incorporation of [3H]palmitate into as and ai and may act to decrease depalmitoylation. Its effect on cAMP responses is too nonspecific to be useful as a tool to study G protein palmitoylation. Treatment with a NO donor slightly decreased palmitoylation of as and ai and caused a marked rightward shift in the isoproterenol dose response curve. This latter effect is unlikely to be due to changes in palmitoylation.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-43011-02

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on a calcium sensing receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen Spiegel, M.D.	Chief	MDB, NIDDK
Others:	P. Goldsmith, Ph.D.	Research Biologist	MDB, NIDDK
	A.Varrault, Ph.D.	Visiting Fellow	MDB, NIDDK
	M. Pena	Visiting Fellow	MDB, NIDDK

COOPERATING UNITS (if any)

Dr. E. Brown, Brigham Hospital, Boston
Dr. F. Fuller, NPS Parmacuticals (proposed CRADA)

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 1.0 | PROFESSIONAL: 1.0 | OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brown and colleagues (Nature 1993) have cloned a novel calcium-sensing receptor (CaR) which is a member of the G protein-coupled receptor superfamily similar in structure to the metabotropic glutamate receptors. The CaR is expressed in a limited range of cell types including kidney, brain, thyroid C cells, and most prominently parathyroid cells. Mutations in the CaR identified in the human genetic disorder familial hypocalciuric hypocalcemia provide strong evidence for the physiologic importance of the receptor in calcium sensing generally and in regulation of parathyroid hormone secretion by extracellular ionized calcium in particular. We are studying several aspects of the receptor's structure and function. We have raised polyclonal antisera to several synthetic peptides corresponding to sequences in the large extracellular domain. These recognize the receptor on western blots and should be useful for a wide variety of studies. Development of monoclonal antibodies is planned with the long-range goal of being used as imaging reagents for parathyroid tumors in patients with failed neck explorations. We are also studying the coupling of the receptor to G proteins using transfection techniques, and by defining the normal complement of G protein alpha subunits in parathyroid cells.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-830

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43204-15 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.E. Balow	Senior Investigator	MDB, NIDDK
Others:	H.A. Austin III	Medical Officer	MDB, NIDDK
	D.T. Boumpas	Visiting Scientist	MDB, NIDDK

COOPERATING UNITS *(if any)*

NIAMS (D. Kastner, J. Klippel, P. Plotz, R. Wilder, C. Yarboro)
CC (E. Vaughan)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20982

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43205-18 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renal Biopsy Pathology in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.E. Balow	Senior Investigator	MDB, NIDDK
Others:	H.A. Austin III	Medical Officer	MDB, NIDDK

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, DC (S. Sabnis); CC (E. Vaughan)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20982

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diverse pathogenetic factors are operant in systemic lupus erythematosus and lead to different forms of lupus nephritis. Detailed analysis of renal biopsy pathology is being conducted on specimens from patients with systemic lupus erythematosus.

Biopsies are classified by standard major category of lupus nephritis, as well as scored on a semi-quantitative scale for specific histologic changes which indicates the extent and severity of active inflammatory lesions and chronic atrophic, fibrosing and sclerosing features. The patterns of immune complex deposition and lymphoid cell interaction with different segments of the nephron are being investigated by immunohistologic techniques and electron microscopy.

These approaches have facilitated the analysis of the effects of various types of immunosuppressive agents used to halt the progression of lupus nephritis and they will enhance our understanding of the pathogenesis of lupus nephritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43208-02 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Halogenated Adenosine Analogs as Immunosuppressive Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	B.J. Fessler	Clinical Associate	MDB, NIDDK
	S. Shrivastav	Chemist	MDB, NIDDK
	H.A. Austin	Medical Officer	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The clinical efficacy, toxicity, and immunological effects of halogenated adenosine analogs, such as fludarabine and chlorodeoxyadenosine, are being studied in patients with autoimmune disorders. These agents primarily affect lymphoid cells because these cells contain an enzyme necessary for activation of these compounds. Previous investigations employing these drugs in patients with low grade lymphoid malignancies have shown that they selectively target immune cells by inducing a physiologic form of cell death called apoptosis. Their effects on the clinical course of immunologic diseases, as well as on the function of immune cells have not been characterized.

Six patients have been entered into a study for treatment of refractory membranous nephropathy. To date, no major or unexpected toxicities have been encountered. Fludarabine treatment has resulted in sustained B and T lymphopenia (B>T). We are attempting to determine whether the level of lymphopenia should be a major determinant of safe drug doses; no substantive changes in total immunoglobulin or antibody levels have been seen. At the low doses of fludarabine used to date, no measurable clinical effects have been noted on the nephropathy. In contrast, significant clinical effects have been observed in eight patients with rheumatoid arthritis who have completed this therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43211-11

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphometry of the Glomerulus in Pimas and other Minority Populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS

COOPERATING UNITS *(if any)*

A Fogo, Dept. Path, Vanderbilt U,Nashville, TN
C M Pesce University of Genova Italy

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, NIH Bdg 10 Room 3 N110 Bethesda MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURALRESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43211-11

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphometry of the Glomerulus in Pimas and other Minority Populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS

COOPERATING UNITS *(if any)*
A Fogo, Dept. Path, Vanderbilt U,Nashville, TN
C M Pesce University of Genova Italy

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, NIH Bdg 10 Room 3 N110 Bethesda MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.00	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43214-11 MD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Glomerular Cells Derived from Normal and Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS
Ci-Jiang HE, Visiting Associate
Zhi-Hong Liu, Guest Worker
O Lenz, Visiting Fellow

COOPERATING UNITS *(if any)*

Phillip Stahl, Washington University
Maria Tsokos, Laboratory of Pathology, NCI

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, NIH Bdg 10 Room 3N110 Bethesda MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.40	1.40	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glomerulosclerosis, increased cell and extracellular matrix turnover, is the leading cause of renal failure in the US. Current studies in models of glomerulosclerosis (GS) have yielded little information about the cellular abnormalities that are critical in the initiation and progression of this disease. This is due, in part, to the difficulty in isolating and characterizing glomeruli in vivo, and the fact that the glomerulus contains three indigenous cell types and a population of bone marrow-derived macrophages. This is particularly a problem with mice, a species we have chosen to study because of the availability of considerable genetic information. We have isolated the individual cell types and characterizing them in vitro. The first series of experiments examined the question of the phenotypic modulation of glomerular mesangial cells in vitro, addressing the question of the effect of differing substrates, the time frame involved, and the molecular consequences of this modulation on extracellular matrix turnover. We found that mesangial cell proliferation and collagen mRNA levels are independently regulated. In addition, the nature of the matrix to which the cells adhered directly affected both proliferation and collagen mRNA levels. The second experiments addressed the handling of macromolecules by mesangial cells, since these cells are capable of ingesting immunoglobulins in vivo. The mannose receptor (MR), a carbohydrate binding membrane protein present on macrophages, was considered to be a candidate for the mechanism by which mesangial cells ingested these large proteins. The MR was found on mesangial cells which had been stimulated with either interleukin-1α or tumor necrosis factor-α.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 43222-10 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Murine Lupus Nephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.:	H. A. Austin III	Medical Officer	MDB, NIDDK
Others:	J. E. Balow	Senior Investigator	MDB, NIDDK
	D. T. Boumpas	Visiting Scientist	MDB, NIDDK

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology; Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work on this project has been suspended during the past year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43224-09 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membranous Lupus Nephropathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.A. Austin III	Medical Officer	MDB, NIDDK
Others:	J.E. Balow	Senior Investigator	MDB, NIDDK
	B.J. Fessler	Clinical Associate	MDB, NIDDK

COOPERATING UNITS *(if any)*

CC (E. Vaughan); Stanford University; Stanford, CA (B. Myers); Armed Forces Institute of Pathology, Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is currently unknown whether therapeutic intervention will alter the course of membranous lupus nephropathy. In the present study, the efficacy and toxicity of three immunosuppressive drug regimens administered over a 12 month period will be evaluated in patients with membranous lupus nephropathy. Detailed tests of renal function (including radiolabelled compounds for glomerular filtration and renal plasma flow rates), glomerular permselectivity (using fractional clearance of graded dextrans) and kidney biopsy morphology will be performed at the beginning and end of treatment. Patients with systemic lupus erythematosus, nephrotic range proteinuria and biopsy documented membranous nephropathy will be randomized to receive: a) alternate day prednisone alone (control group), b) alternate day prednisone plus intravenous pulse cyclophosphamide up to 1.0 gram per square meter body surface area every other month for 6 total doses, or c) alternate day prednisone plus oral cyclosporin A up to 200 mg per square meter body surface area daily. Lupus disease activity, renal function tests and drug toxicities will be monitored closely. Analysis will include comparison of the numbers of favorable outcomes of glomerular filtration rate, renal plasma flow, permselectivity, glomerular pathology and drug-related toxicities appearing in each treatment group. To date, response rates have been higher in patients receiving cyclophosphamide or cyclosporin A than in those receiving corticosteroids alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43225-08

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glomerular Lesions in Mice Transgenic for Growth Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
Oliver Lenz, Visiting Fellow
CW Yang, Visiting Associate
ZH Liu, Guest Worker

COOPERATING UNITS *(if any)*

John Kopchick, University of Ohio

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology, Bldg. 10, Rm. 3N-110

INSTITUTE AND LOCATION

NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.80	PROFESSIONAL: 0.80	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously reported that mice transgenic for growth hormone develop a rapidly progressive glomerulosclerosis. In contrast, mice transgenic for a mutated growth hormone (G 119K) have a dwarf phenotype and have normal glomeruli. We postulated that growth hormone was required for the appearance of glomerulosclerosis and that the dwarf mice would be protected from diabetic renal lesions. We made normal non-transgenic mice diabetic by using repeated streptozotocin injections. The mice were kept alive 3 months without insulin treatment. The glomeruli showed marked hypertrophy and diffuse mesangial lesions with appearance of nodules characteristic of diabetic nephropathy. The dwarf transgenic mice were also made diabetic but failed to develop glomerular hypertrophy and glomerular lesions. These data show that the GH antagonist which is expressed at high levels in the transgenic mice completely prevents the development of diabetic glomerulosclerosis. Furthermore, immunofluorescence microscopy showed accumulation of type IV collagen and laminin in the control, but not in the transgenic mice. Competitive PCR of microdissected glomeruli was performed in order to examine genes coding for laminin B1, α1 Type IV collagen and showed that they were elevated in the control but not in transgenic mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43228-08

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Human Mesangial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: GE Striker, Senior Investigator
Others: LJ Striker, Chief RCBS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK NIH Bethesda, Md 20892, Bldg 10 Room 3N-110

TOTAL STAFF YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☒ (b) Human ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is currently inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43231-07 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Idiopathic Membranous Nephropathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	H.A. Austin III	Medical Officer	MDB, NIDDK
Others:	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

Stanford University, Stanford, CA (B. Myers); CC (E. Vaughan); Armed Forces Institute of Pathology; Washington, DC (S. Sabnis)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with idiopathic membranous nephropathy are being studied to evaluate the efficacy and toxicities of the addition of intermittent cyclophosphamide to alternate day oral corticosteroid therapy. Efficacy will be judged by determinations of effective renal plasma flow, glomerular filtration rate and glomerular capillary wall permselectivity performed with dextran and urine protein (albumin and immunoglobulin) clearance techniques. Kidney biopsy morphology (including morphometric analysis) will be examined at the beginning and at the end of treatment as part of detailed studies of structure-function relationships and the efficacy of various therapeutic modalities.

Patients with membranous nephropathy and 2 or more grams per day of proteinuria will be treated with alternate day prednisone and will be randomized to receive: a) no additional therapy (control group), or b) intravenous pulse cyclophosphamide up to 1.0 gram per square meter body surface area every other month for 6 total doses. Analysis will include comparison of the number of favorable outcomes of glomerular function and pathology, as well as drug-related toxicities observed in each treatment group at the end of the 12 months of study.

To date, the rates of renal outcomes are not significantly different in the groups receiving prednisone alone or prednisone plus intermittent cyclophosphamide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43232-06 MD

PERIOD COVERED

October 1, 1994 to September 30, 1995.

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Renal Lesions in the Ablation Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: LJ Striker, Chief, RCBS

Others: GE Striker, Senior Investigator

COOPERATING UNITS (if any)

Saulo Klahr, Washington University, St. Louis, MO.
Carlo Pesce, Dept. of Pathology, Genova, Italy

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bethesda, MD 20892, Bldg. 10, Room 3N-110

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43235-06

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glomerular Lesions in Non-Obese Diabetic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
C W Yang, Visiting Associate
Ci Jiang He, Visiting Associate
Zhi Hong Liu, Special Volunteer

COOPERATING UNITS *(if any)*

M Hattori, Joslin Institute, Harvard Medical School, Boston, MA

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40	0.40	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NOD mice spontaneously develop insulin-dependent diabetes mellitus (IDDM) secondary to immunologically-mediated beta cell destruction in pancreatic islets. These mice exhibit an increase in the amount of mesangial matrix before diabetes mellitus occurs and are therefore "prone to glomerulosclerosis". Shortly after the appearance of diabetes, there is an accentuation of the renal lesions consisting of mesangial sclerosis, thickening of glomerular basement membranes, and albuminuria. Morphometric analysis showed that the kidney weight and glomerular size were increased in diabetic mice, compared to non-diabetic mice, and that the ratio glomerular volume/kidney weight was elevated in diabetic mice. We explored by competitive PCR of isolated glomeruli the glomerular turnover of the extracellular matrix components. Prior to the appearance of diabetes the levels of type IV collagen mRNA were 3-4 times higher than in SJL mice who have normal glomeruli. In addition the NOD mice had fewer glomeruli than the SJL and those were larger. Therefore the NOD strain has a propensity to develop sclerosis irrespective of diabetes which is underlain by an abnormal level of expression of basement membrane gene expression and by a reduced number of glomeruli. Shortly after the occurrence of diabetes mellitus we found an upregulation of laminin but not collagen gene expression. This was associated with an increase in TGF-ß1 gene expression which may represent one of the early molecular events in diabetic nephropathy. We also found that there was accumulation of the advanced glycosylation end products in the kidneys shortly after the onset of diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43236-05

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glomerular Effects of Advanced Glycosylation End Products(AGEs)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: CW Yang, Visiting associate
GE Striker, Senior investigator

COOPERATING UNITS *(if any)*

Helen Vlassara, Picower Institute for Medical Research, Manhasset NY

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bdg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.10	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

End-stage glomerulosclerosis constitutes a major complication of diabetes mellitus. The fact that the glomerular lesions in both IDDM and NIDDM are similar suggests that abnormalities in glucose metabolism may participate in their development. Hyperglycemia leads to the accumulation of advanced glycosylation end-products. These products participate in abnormal, non-metabolizable cross-linking of extra-cellular matrix components. Their accumulation may contribute to the sclerosis observed in diabetics. AGEs trigger a large number of biological reactions which are mediated by surface receptors that have been characterized on macrophages, endothelial cells, and human and rat mesangial cells. Using normal mouse mesangial cells, we showed that cells exposed to AGE had increased levels of the following mRNAs using the RNAse protection assay: collagen type IV, proteoglycan heparan sulfate, and laminin A and B chains. We also found an increased release of collagen type IV into the medium. We explored the effects of injections of AGEs to the intact animal would have similar effects. normal rats receiving long-term injections of AGE-Albumin had albuminuria and developed focal sclerotic glomerular lesions. The coadministration of aminoguanidine which inhibits cross linking of AGEs prevented the renal lesions. We also found that the glomeruli of normal mice receiving repeated injections of AGEs exhibited an increase in mRNAs coding for α1 type IV collagen and for the B1 chain of laminin establishing that there is a glomerular response to AGEs in vivo. These responses were associated with an increase in TGF-ß1 but not PDGF message level. These responses did not occur when the mice were cotreated with aminoguanidine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43237-04

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of Metalloproteinases and TIMPs by Mouse Glomerular Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: G Striker, Senior Investigator
T. Tamaroglio, Fellow (IRTA)

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg. 10, Rm. 3N-110, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.20	1.20	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ © Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glomerulosclerosis occurs in a large number of human kidney diseases, including diabetic nephropathy. It consists of the accumulation of extracellular matrix (ECM) within the glomerulus. Sclerosis may be mediated by dysregulation of both synthesis and degradation of ECM. A large family of matrix metalloproteinases as well as tissue inhibitors of metalloproteinase (TIMPs) play a role in the degradative process. Our preliminary work reveals that: 1) normal mouse mesangial cells in culture secrete a 72 kD and a 92 kD gelatinase (type IV collagenase) as well as TIMP1; 2) mesangial cells derived from NOD mice and mice transgenic for bovine growth hormone (bGH) secrete mostly the 72 kD gelatinase; Normal mouse glomeruli contain mRNA coding the 72kD species. This mRNA is increased in mice transgenic for bGH who have sclerotic lesions. TIMP mRNAs are undectable in mouse glomeruli which emphasizes the major phenotypic changes that occur in vivo. In the GH mice the upregulation of the 72KD gelatinase is less prominent than that of type IV collagen. This finding may suggest that there is an imbalance between synthesis and degradation of extracellular matrix in certain forms of glomerulosclerosis. 92kD gelatinase is also being investigated at the mRNA and protein levels, since it appears to increase in response to exposure to pentosan sulfate. This may have important therapeutic applications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43238-05

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression in Microdissected Mouse Glomeruli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L Striker, Chief RCBS
Others: G Striker, Senior Investigator
O Lenz, Fellow
CJ He, Visiting Associate
Zhi-Hong Liu, Special Volunteer

COOPERATING UNITS *(if any)*

R Zalups PhD, Department of Physiology, Macon, Georgia
Anthony Wynshaw-Boris, MD, PhD, LGDR, NCHGR

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, Bldg 10, Room 3N-110, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.90	0.90	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to determine the phenotype of multiple extracellular matrix molecules in adult mouse glomeruli from different strains of mice. We used the increased sensitivity afforded by the polymerase-chain reaction to assess α1 type I and several α chains of type IV collagen mRNA as well as laminin subchains in freshly microdissected normal adult mouse glomeruli. RT-PCR reactions for mRNA encoding these components were also performed using mesangial cell lines previously isolated from the same strain of mice. Type IV collagen mRNA was easily detectable in normal adult mouse glomeruli as well as in the cell lines. Assays using competitive PCR were developed. The current study provides evidence that the expression of types I and IV collagen in normal glomeruli is regulated at the pretranslational level in vivo. We have applied this method to a mouse strain which develops spontaneous glomerulosclerosis, the ROP-OS mouse. This mouse is originally derived from a radiation-induced mutation. Our preliminary data indicate that the mutation induces a rapid glomerulosclerosis on a ROP background but has little effect on a C57Bl background. The ROP-OS mouse has a 3 fold increase in mRNAs coding for type IV collagens and laminin early in life at a time when morphologic evidence of glomerulosclerosis is quite discrete. This model allows the study of ureteric bud branching in renal development, and the relationships between glomerular number, size and sclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43239-04 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms and Modulation of T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	N. Hama	Visiting Fellow	MDB, NIDDK
	F. Paliogianni	Courtesy Associate	MDB, NIDDK
	B.J. Fessler	Clinical Associate	MDB, NIDDK
	S. Shrivastav	Chemist	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

University of Washington, Seattle, WA (M.A. Valentine)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activation of T cells is a complex process involving cell membrane, cytoplasmic and nuclear events. These events enable T cells to proliferate and exert their immunoregulatory function.

The purpose of these studies is to define the effects of biologic agents used for the therapy of immune mediated renal diseases (glucocorticoids, cyclosporin A), as well as those produced at the site of inflammation (prostaglandin E_2, transforming growth factor ß), on molecular events associated with T cell activation. T cell activation through it antigen specific receptor results in increased intracellular calcium and activation of protein kinase C (PKC). Increased intracellular calcium, in turn, activated calmodulin (CaM) dependent kinases, such as CaM kinase II and phosphatases, such as calcineurin.

Glucocorticoids downregulate the activity of CaM kinase II, but not PKC, during T cell activation by upregulating protein phosphatases 1 and/or 2A, but not 2B. Both cyclic AMP elevating agents and glucocorticoids inhibit calcineurin-dependent pathways for T cell activation (calcineurin being a calcium/calmodulin-dependent 2B phosphatase).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43241-04

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Pathogenesis of Glomerulosclerosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
C Esposito, Special Volunteer
CJ He, Visiting Associate

COOPERATING UNITS *(if any)*
International Collaborative Group for Molecular Studies of Kidney Biopsy (See list below)

LAB/BRANCH
Metabolic Diseases

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, Bldg. 10, Rm. 3N-110, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☒ (b) Human ☐ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most progressive glomerular diseases leading to endstage renal disease have increased extracellular matrix (ECM) within mesangial areas. Elucidation of the nature and the rate of ECM accumulation in diseased states are prerequisites for understanding, and possibly interfering with, progressive glomerulosclerosis. Changes in whole kidney preparations do not accurately reflect those in glomeruli. For these reasons we developed a technique consisting of microdissection of glomeruli, in situ RT of mRNA, and PCR. To quantitate changes in type IV collagen mRNA expression, we developed a competitive PCR assay. We first examined nephrectomies performed for renal carcinoma, since nearly 50% of these patients have glomerulosclerosis. We found that α2IV collagen cDNA was significantly elevated in patients with glomerulosclerosis. There was not a parallel increase in cell number, suggesting that the increment was due to upregulation of α2IV collagen mRNA, rather than cellular proliferation. As we found in mice, glomerular α2IV collagen cDNA level remained high, independently of the degree of sclerosis, even in patients who had extensive glomerular scarring. Satisfied that we had the appropriate tools to examine the pathogenesis of renal disease in humans, we started a multi-center collaboration to develop markers of progression. We obtained either cDNA or isolated microdissected glomeruli from diagnostic renal biopsies. The initial focus is on diabetes mellitus and IgA nephropathy. Our preliminary results indicate that the α2/α3 type IV collagen ratio is equal to 1 in normal kidneys, is high in diabetic nephropathy, and is 1 in membranous glomerulonephritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURALRESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43242-04

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Degradation of Extracellular Matrix in Human Glomeruli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: LJ Striker, Chief RCBS
Others: GE Striker, Senior Investigator
C Esposito, Visiting Guest

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases

SECTION

Renal Cell Biology

INSTITUTE AND LOCATION

NIDDK, NIH Bdg 10 Room 3N110 Bethesda MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.15	0.65	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ © Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glomerulosclerosis is characterized by a net accumulation of extracellular matrix components in the glomerulus and results in its progressive obliteration. We started to investigate whether this condition could result from an imbalance between synthesis and degradation of the extracellular matrix. The metalloproteinases which degrade basement membrane collagens are inactivated by a series of inhibitors: TIMPs (tissue inhibitors of metalloproteinases). We examined whether TIMPs mRNAs and the corresponding peptide were expressed in normal and sclerotic glomeruli. We utilized the non tumoral part of nephrectomies performed for cancer in a series of 10 adult patients. The glomeruli were microdissected and reverse-transcribed in situ. Competitive polymerase chain reaction was performed for TIMP I and II mRNAs. Both were detected in the glomeruli and were increased in the specimens in which glomerulosclerosis was present. The increase was not due to an increase in the number of cells in the glomeruli with sclerosis. These data suggest that TIMPs are increased on a per cell basis in the glomerulosclerosis associated with carcinoma. Further studies are in progress to determine whether this increase is also observed in other forms of human glomerulosclerosis. We have also obtained preliminary data indicating that mRNA coding for the 72kD metalloproteinase is expressed in human glomeruli. This species appears to undergo the same upregulation than the $\alpha 2$ chain of type IV collagen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43243-03 MD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Co-stimulatory Signals for T-Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.T. Boumpas	Visiting Scientist	MDB, NIDDK
Others:	B.J. Fessler	Clinical Associate	MDB, NIDDK
	F. Paliogianni	Courtesy Associate	MDB, NIDDK
	N. Hama	Visiting Fellow	MDB, NIDDK
	J.E. Balow	Senior Investigator	MDB, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Experimental Immunology, FCRP, NCI (H.A. Young)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecules that interact with the antigen-specific receptor on T-cells, including peptides bound to major histocompatibility complex (MHC) molecules, antibodies against the antigen specific receptor, and mitogenic lectin, fail on their own to stimulate T cells to proliferate. At least one additional signal is required. This second signal is referred to as co-stimulation, and it is delivered by antigen-presenting cells.

The molecular nature of co-stimulation has been sought for many years in order to facilitate therapeutic strategies for suppressing immune responses. The goal of these studies is to dissect further the molecular mechanisms of co-stimulation by the use of naturally occurring immunosuppressive agents.

Findings to date indicate that glucocorticoids inhibit activation of NF-AT and IL-2Rα expression but not the tyrosine phosphorylation and calcium influx when cells are stimulated via CD2. IL-2 production is inhibited by glucocorticoids in cells treated with phorbol ester and anti-CD28.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43300-02-MDB

PERIOD COVERED
October 1, 1994 to Septmber 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular biologic studies on the cause of parathyroid neoplasia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen Spiegel, M.D.	Chief	MDB/NIDDK
Others:	D. Wenkert, M.D.	Clinical Associate	MDB, NIDDK
	Q. Dong, M.D., Ph.D.	Visiting Fellow	MDB, NIDDK

COOPERATING UNITS (if any)
S. Marx, M.D., Chief, Genetics and Endocrinology Section, MDB, NIDDK
F. Collins, M.D., Ph.D., NCHGR

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Primary hyperparathyroidism (HPT), a common endocrine disorder that can cause significant morbidity, may be due to benign neoplasia of a single parathyroid gland (adenoma) or multiple parathyroid glands (hyperplasia), and rarely, to malignant neoplasia of a parathyroid gland (carcinoma). The etiology of parathyroid neoplasia has not been defined. As with other forms of neoplasia, parathyroid tumors are presumably due to inherited (germ-line mutation) and/or acquired (somatic mutation) defects in specific genes. Etiologic genetic defects could include inappropriate expression of transforming "oncogenes" and/or loss of expression of tumor "suppressor" genes. The availability of surgically resected parathyroid tumors from patients with sporadic and hereditary forms of disease allows us to search for tumor-specific genetic abnormalities that may be involved in development of parathyroid neoplasia.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43301-02 MDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Guanine nucleotide binding proteins as receptor-effector couplers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen Spiegel, M.D.	Chief	MDB, NIDDK
Others:	A. Shenker, M.D.Ph.D.	Senior Research Investigator	MDB, NIDDK
	Q. Dong, M.D., Ph.D.	Visiting Fellow	MDB, NIDDK
	P. Goldsmith, Ph.D.	Research Biologist	MDB, NIDDK

COOPERATING UNITS (if any)
S. Kosugi, University of Kyoto, Japan

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 2.5 | PROFESSIONAL: 2 | OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A family of guanine nucleotide binding proteins (G-proteins) functions in transmembrane signalling as receptor-effector couplers. G-proteins couple to a diverse array of receptors including those for hormones, neurotransmitters, light, odorants, and certain growth factors. Effector functions regulated (positively and, in some instances, negatively) by G-proteins include cAMP formation, phosphoinositide breakdown, potassium and calcium channels, and cGMP degradation. We have used a variety of techniques to study the expression, distribution, regulation, structure and function of G-proteins. Our studies highlight the diversity within the G-protein family. Using peptide specific antibodies, we have defined the specificity of G-proteins in coupling to receptors and effectors. We have created mutations in alpha subunits that cause constitutive activation, and transfected these into cells to define phenotypic effects on cellular function. We have identified mutations in G proteins and in G protein-coupled receptors as the basis of several human diseases. These studies provide the basis for understanding the role of G-proteins in normal signal transduction and for elucidating possible defects in G-protein structure or function as the basis for abnormal signal transduction.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DK 43302-02 MDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Pseudohypoparathyroidism and Related Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Weinstein, M.D.	Senior Staff Fellow	MDB, NIDDK
Others:	Shu-hua Yu, Ph.D.	Visting Associate	MDB, NIDDK
	Dawen Yu, Ph.D.	IRTA	MDB, NIDDK

COOPERATING UNITS (if any)
H. Westphal E. Lee, LMGD, NICHD, Bethesda, MD
M. Nanes, V.A. Medical Center, Emory University, Decator, GA.
E. Schipani, H. Juppner, Endocrine Unit, Massachusetts General Hospital, Boston, MA.

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS 3.5	PROFESSIONAL: 3	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1942 Albright described the features of an inherited clinical syndrome named "pseudohypoparathyroidism" (PHP). The biochemical hallmark of PHP is a lack of response of urinary cyclic AMP (cAMP) to exogenous parathyroid hormone (PTH). This indicates that there is a defective hormone receptor-adenylyl cyclase complex in this disorder. Patients with one form of PHP show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and resistance to multiple hormones including PTH,thyrotropin and gonadotropins (PHP Ia). Other affected relatives from PHP Ia kindreds have AHO without hormone resistance (pseudopseudo-hypoparathyroidism, PPHP). We have shown that many patients with PHP Ia and PPHP show an approximately 50% reduction in activity of Gs (the stimulatory guanine nucleotide binding protein associated with adenylyl cyclase) in membranes from multiple tissues. We have shown that steady state Gs-alpha mRNA levels from fibroblasts of subjects with PHP Ia and PPHP are reduced by approximately 50% compared with normals and have identified genetic abnormalities which account for Gs deficiency. It has recently been suggested that the same genetic defect in the Gs-alpha gene may result in PHP Ia or PPHP by a mechanism called parental imprinting. Another form of PHP is characterized by isolated PTH resistance without features of AHO or resistance to other hormones (PHP Ib). Previous work in this laboratory showed a selective resistance of cAMP generation to PTH in fibroblasts from these patients, suggesting a potential defect in the PTH receptor. Molecular studies to date suggest that these are not due to mutations in the coding exons of the PTH receptor gene. There are also a small number of patients who have AHO and generalized hormone resistance but who do not have a Gs defect (PHP Ic). The molecular defect in these patients have not as yet been identified.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43303-02 MDB

PERIOD COVERED
October 1, 1994 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on McCune-Albright Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen Spiegel, M.D.	Chief	MDB, NIDDK
Others:	A. Shenker, M.D., Ph.D.	Senior Researcher Investigator	MDB, NIDDK

COOPERATING UNITS (if any)
P. Marie, Ph.D. INSERM -Paris, France

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 0.5
PROFESSIONAL: 0.5
OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

McCune-Albright syndrome (MAS) is a non-inherited disorder in which affected subjects show a variety of seemingly unrelated abnormalities including polyostotic fibrous dysplasia, pigmented skin lesions (cafe-au-lait spots), and autonomous hyperfunction of various endocrine organs including gonads, anterior pituitary, thyroid, and adrenal cortex. The endocrine abnormalities lead to precocious puberty, gigantism/acromegaly, hyperthyroidism, and hypercortisolism. The cause of this sporadic disorder has been enigmatic, but speculations have centered on a defect in signal transduction leading to endocrine hyperfunction. The distribution of skin lesions has also suggested the possibility of a somatic mutation acquired early in embryogenesis and affecting only a subset of cells (mosaicism). Since a G protein mutation could plausibly explain the endocrine manifestations, we searched for and found mutations of the Gs-alpha gene that lead to constitutive activation of the Gs protein. These mutations were found in a mosaic distribution; notably, mutant gene was undetectable in normal-appearing portions of endocrine glands, but was present at heterozygous levels in neoplastic portions of endocrine tissue. Mutant Gs-alpha was also detected in dysplastic bone lesions, both in the polyostotic, "classical" form of MAS and in a "form fruste" of the disease, monostotic fibrous dysplasia. Occurrence of mutant Gs-alpha in organs such as heart and liver suggest a possible role in "non-classical" manifestations, including sudden death. Our studies suggest that MAS is caused by a somatic mutation in the Gs-alpha gene occurring early in development and found in a mosaic distribution.More focal manifestations of the disease such as monostotic fibrous dysplasia may be caused by somatic mutation of the Gs-alpha gene occuring later in development.

U S GOVERNMENT PRINTING OFFICE. 1991 0-864-809

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 DK 43304-02 MDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Guanine nucleotide binding protein beta-gamma dimers: structure and function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William F. Simonds, M.D.	Senior Clinical Investigator	MDB, NIDDK
Others:	Chunghee Lee, Ph.D.	Visiting Associate	MDB, NIDDK
	Shiying Zhang, Ph.D.	Visiting Fellow	MDB, NIDDK
	Susan Pellegrino, Ph.D.	IRTA Fellow	MDB, NIDDK

COOPERATING UNITS (if any)
J. Silvio Gutkind, Ph.D. LCDO, NIDR
R. Collins, Ph.D., MDB, NIDDK
A. Garritsen, Ph.D. Organon International, The Netherlands (Foreign)

LAB/BRANCH Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 3.25 | PROFESSIONAL: 3.25 | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The guanine-nucleotide binding regulatory proteins (G-proteins) are $\alpha 1 \beta 1 \gamma 1$ heterotrimers which function as transmembrane signal transducers by coupling receptors for extracellular stimuli to intracellular effectors (enzymes, ion channels). G-proteins constitute a diverse family distinguished by specific receptor and effector interactions which in turn are determined by the structure of the three constituent subunits. The α subunit binds guanine nucleotides and has a well established role in effector modulation. The β and γ subunits are tightly associated as a βγ complex, comprising a single functional entity which, like the α subunit, is absolutely required for G-protein interaction with receptor. An effector modulatory role for the βγ complex is becoming increasingly apparent in several systems. The present research emphasizes the role of the βγ complex in G-protein-mediated signal transduction. We have used subunit specific peptide antibodies to probe regions of the βγ complex important for functional interaction with the α subunit and to monitor expression of recombinant subunits. Site-directed mutagenesis has been used to study the assembly, processing and effector function of the βγ complex in both transient and stable transfected cell systems. These studies may elucidate the contribution of the βγ subunit complex to the receptor and effector selectivity characteristic of G-proteins and to the adaptive responses pursuant to agonist stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 43305-02 MDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on nephrogenic diabetes insipdus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen Spiegel, M.D.	Chief	MDB, NIDDK
Others:	D. Wenkert, M.D.	Clinical Associate	MDB, NIDDK
	M. Pena, M.D.	Visiting Fellow	MDB, NIDDK

COOPERATING UNITS (if any)

J. Wess, Ph.D., LBC, NIDDK

LAB/BRANCH Metabolic Diseases Branch

SECTION Molecular Pathophysiology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD

TOTAL STAFF YEARS 0.75 PROFESSIONAL: 0.5 OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nephrogenic diabetes insipidus (NDI) is an inherited X-linked disorder in which affected subjects are resistant to the actions of vasopressin (AVP) on renal medullary cells responsible for water concentration. Clinical manifestations include severe polydipsia and polyuria, and resultant severe dehydration can lead to cerebral swelling and death. Treatment with a potent AVP analog (DDAVP), useful in other forms of DI, is ineffective in NDI because of end-organ resistance to the hormone. The renal actions of AVP are mediated through a V2 type receptor linked via the Gs protein to stimulation of the 2nd messenger cAMP. In theory, the inherited gene defect could be located anywhere along the signal transduction path, but indirect evidence suggested a likely receptor defect. The recent cloning of a human V2 receptor permitted chromosomal localization studies which showed that the receptor is localized to Xq28, the site of the gene defect as determined by family linkage studies. This strongly suggested but did not prove that a receptor gene mutation is the underlying defect in NDI. We have obtained genomic DNA samples on multiple families with NDI, and in eight families thus far have identified mutations predicted to disrupt formation of a normal V2 receptor. These findings have important implications for our understanding of the pathogenesis of NDI and of normal V2 receptor structure and function, for identification of affected subjects and carriers, and eventually for gene therapy of the disease.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-600

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43306-01 MDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

TGF-beta Superfamily Molecules in Renal Development and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jeffrey Kopp	Senior Clinical Investigator	MDB, NIDDK
Others:	Wataru Kajiyama	Visiting Associate	MDB, NIDDK
	Miklos Mozes	Visiting Fellow	MDB, NIDDK
	Shashi Shrivastav	Chemist	MDB, NIDDK
	Slobodan Vukicevic	Courtesy Associate	MDB, NIDDK

COOPERATING UNITS (if any)

Dr. Snorri Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI

LAB/BRANCH Metabolic Diseases Branch

SECTION Kidney Disease Section

INSTITUTE AND LOCATION NIDDK

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.8	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The TGF-beta superfamily includes the bone morphogenic proteins (BMP), which were initially isolated from bone and defined by their ability to induce bone formation when implanted subcutanteously. The expression of BMPs in tissues other than bone, and in particular the abundant expression of BMP-3, BMP-4, and BMP-7 (osteogenic protein-1, OP-1) in kidney, suggest that they may have other important actions. In the developing kidney, OP-1 is produced by the advancing ureteric bud and by mesenchymal cell condensates. We have examined the role of OP-1 in organ culture of mouse metanephric rudiments from embryonic day 11.5 (prior to penetration by ureteric bud and therefore composed of uninduced mesenchyme) and embryonic day 12.5 (after penetration by ureteric bud and therefore showing mesenchymal transition to epithelial tissue). We have found that 1) the addition of recombinant soluble OP-1 to day 11.5 metanephroi induces undifferentiated mesenchyme to undergo conversion to epithelium and 2) the addition of either neutralizing antibody or antisense to OP-1 to day 12 metanephroi antagonizes epithelial differentiation. These findings support a role for OP-1 in inducing the mesenchymal to epithelial transformation, and further suggest that OP-1 may play a critical, previously unrecognized role in renal development.

Local, intra-renal TGF-beta 1 production is a well-recognized mediator in experimental animal models of chronic renal disease. We have identified and characterized a renal syndrome caused by elevated plasma levels of TGF-beta 1, using a mouse transgenic for TGF-beta 1 under the control of the albumin promoter, in which transgene expression is confined to the liver. These mice have plasma TGF-beta 1 levels which are 8-fold elevated at 3 weeks of age. Renal disease is notable for activation of the glomerular endothelial cell and expansion of the mesangium, present by 3 weeks of age. There is accumulation of extracellular matrix within the glomerulus and interstitium, including collagens I and III. Northern analysis indicates that this accumulation is due to a combination of increased mRNA for collagen I and III and the proteoglycans biglycan and decorin and a striking increase in TIMP-1 mRNA. These findings suggest that circulating levels of TGF-beta can induce severe renal disease, at least in part by altering matrix remodelling within the kidney. We are testing the ability of TGF-beta 1 antagonists to retard renal disease in this model, including neutralizing antibody.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 47001-13 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Insulin-like Growth Factor-1 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: D. LeRoith Medical Officer, (Res.) E. Karnieli, Guest Researcher
Others: V. Blakesley, Clinical Associate
B. Stannard, Biologist
H. Werner, Visiting Scientist
D. Beitner-Johnson, PRAT Fellow
C. Hernandez-Sanchez, Visiting Fellow
A. Koval, Visiting Fellow
A. Scrimgeour, Guest Researcher

COOPERATING UNITS *(if any)*
C.T. Roberts, Jr. Oregon Health Sci. Univ. (Portland, OR)
Y. Zick Weizmann Institute (Rehovot, Israel)

LAB/BRANCH
Diabetes Branch

SECTION
Section on Molecular and Cellular Physiology

INSTITUTE AND LOCATION
National Institute of Diabetes and Digestive and Kidney Diseases

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.5	7.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Insulin-like growth factor-I receptor (IGF-IR), like the insulin receptor, is a tyrosine kinase transmembrane glycoprotein. Expression of the IGF-IR is highly regulated during development and in certain disease states including diabetes and cancer. The activity of the IGF-IR promoter is regulated by various growth factors and nuclear transcription factors. Whereas, IGF-I inhibits the expression of its own receptor, fibroblast growth factor (FGF) enhances expression via a mechanism which involves enhancement of the promoter. Tumor suppressor gene products, including WT-1 and p53, inhibit the promoter activity of the IGF-IR. Mutant tumor suppressors, on the other hand, lost their ability to suppress the IGF-IR promoter. These results may explain the increased levels of IGF-I receptors in many cancers expressing mutant forms of these tumor suppressor gene products.

Mutational analysis of the IGF-IR has shed light on the structural and functional aspects of this tyrosine kinase receptor. Mutation of the tyrosine kinase domain results in loss of all IGF-I-induced functions including the ability of this receptor to induce tumors in nude mice. Deletion of the C-terminus region of the receptor, though resulting in a moderate decrease in IGF-I-induced functions such as mitogenesis, results in a loss in tumorigenicity. Substitution of the double tyrosines (1250, 1251) in the C-terminus similarly inhibits tumorigenesis. Thus, the ability of the IGF-IR to induce tumors seems to emanate, at least partially, from the C-terminal region of the receptor beta subunit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK47005-23 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of insulin receptors in circulating cells in man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P. Gorden	Director	NIDDK
Others:	C. Hendricks	Biol. Lab. Tech.	DB, NIDDK
	P. Roach	Senior Staff Fellow	DB, NIDDK
	P. Formisano	Special Volunteer	DB, NIDDK
	S.I. Taylor	Chief	DB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Merged into Project No. Z01 47022 - 16 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

/Z01 DK 47007-19 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Antibodies to Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: S. Taylor, Chief, DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Receptors & Hormone Action

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47018-17 DB

PERIOD COVERED
October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Insulin-Like Growth Factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.'s:	D. LeRoith	Section Chief
Others:	S. Neuenschwander	Visiting Fellow
	A. Koval	Visiting Fellow
	Z. Shen-Orr	Guest Researcher
	D. Landau	Guest Researcher

COOPERATING UNITS (if any)
C.T. Roberts, Jr., Oregon Health Sci. Univ., Portland, Oregon
V. Kavsan Kiev, Ukraine

LAB/BRANCH
Diabetes Branch

SECTION
Section of Molecular and Cellular Physiology

INSTITUTE AND LOCATION
National Institute of Diabetes, Digestive and Kidney Diseases, NIH 10/8D48

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Summary The insulin-like growth factor (IGF) family comprises three peptides, (insulin, IGF-I and IGF-II), six binding proteins (IGFBPs 1-6) and two receptors with inherent tyrosine kinase activity (insulin and IGF-I receptors), and one receptor with no known signalling function (IGF-II/M-6-P receptor). They are all widely expressed and play an essential role in growth and development of all tissues, as well as in the normal functioning of differentiated tissues.

The gene for IGF-I is highly conserved throughout vertebrate evolution; the salmon IGF-I gene encodes a peptide very similar to mammalian IGF-I. The biological actions of IGFs in mammalian tissues are modulated by locally produced IGFBPs. Cancer cells express IGFBPs in different combinations. Human salivary gland cancers express IGFBPs 3,4,5, whose release from the cell is regulated by growth factors and cytokines including interferon and tumor necrosis factor. These alterations in IGFBP release may mediate the inhibitory action of these cytokines on cancer cells. During the early stages of diabetic kidney disease there are hemodynamic changes that are partially induced by IGF-I which accumulates in the kidney due to increased IGFBP expression. Long-term disease is also associated with alterations in expression of certain IGFBPs and these changes may be associated with the glomerular basement membrane thickening seen at this stage of the disease.

The IGFs also play an important role in the immune system. IGF administration to rodents and monkeys induces changes in the immune system including splenic enlargement and increased % splenic T cells. Thus, the IGFs (in combination with growth hormone) may prove to be useful agents in certain immune deficiency syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47019 19 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocytosis and intracellular trafficking of insulin receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Phillip Gorden	Director, NIDDK
Others:	Simeon I. Taylor	Chief, DB, NIDDK
	Carol Renfrew Haft	IRTA, DB, NIDDK
	Maria de la Luz Sierra	Biologist, DB, NIDDK

COOPERATING UNITS *(if any)*

Richard Klausner, Child Health, CBMB; Jean-Louis Carpentier, Univ. of Geneva, Switzerland.

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When insulin binds to the insulin receptor, this stimulates endocytosis of the hormone-receptor complex. Ligand-stimulated endocytosis requires that the receptor must possess intact tyrosine kinase activity. In this project, we are undertaking to identify other sequence motifs in the intracellular domain of the insulin receptor that are required for endocytosis. Dileucine motifs have been shown to be involved in trans Golgi sorting, lysosomal targeting, and internalization of a number of proteins. Because the insulin receptor contains four dileucine pairs in its cytoplasmic domain, we investigated whether these insulin receptor sequences could serve as lysosomal sorting sequences. Chimeric molecules expressing the Tac antigen fused to each isolated insulin receptor motif were constructed. A chimera containing the juxtamembrane dileucine motif (Glu-Lys-Ile-Thr-Leu-Leu), which closely resembles the sequences originally identified in the gamma- and delta-chains of the T cell receptor, was shown to sort to lysosomes by immunofluorescence microscopy, as did a chimera expressing one dileucine motif (Gly-Gly-Lys-Gly-Leu-Leu) found in the tyrosine kinase domain. Chimeras expressing two other dileucine motifs localized to both lysosomes and the plasma membrane. In contrast, chimeras expressing two other potential sorting signals found in the cytoplasmic tail of the insulin receptor (Asn-Ala-Arg-Asp-Ile-Ile and Lys-Asn-Gly-Arg-Ile-Leu) localized predominantly to the plasma membrane. When alanine residues were substituted for the two leucines in the Glu-Lys-Ile-Thr-Leu-Leu sequence, the resultant chimera was localized exclusively to the cell surface. When the alanine pair was substituted for the two leucine residues in the juxtamembrane domain of the intact insulin receptor and the mutant receptor was expressed in NIH-3T3 cells, the mutation did not impair insulin binding or receptor tyrosine kinase activity. However, the Ala-Ala mutant internalized insulin 80% slower than the wild-type receptor. Taken together, these findings suggest that the dileucine motif found in the juxtamembrane domain of the insulin receptor is involved in receptor internalization. Apparently, other dileucine motifs in the insulin receptor do not completely replace this function of the juxtamembrane dileucine motif. Thus, it is likely that other insulin receptor sequences mask the other potential lysosomal targeting signals in the intact molecule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 47022-16 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Molecular genetics of insulin resistance and noninsulin-dependent diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: S.I. Taylor, Chief, DB, NIDDK

Others: D. Accili Visiting Scientist, DB	Y. Imai Vositing Assoc., DB
M. Lesniak, Chemist, DB	L. Sierra, Biologist, DB
P. Roach, Clin. Assoc., DB	M. Quon, Clin. Assoc., DB
E. Wertheimer, Visiting Fellows, DB	C.R. Haft, IRTA, DB
R. L. Toledano, Visiting Assoc., DB	L. Sorbara, SSF, DB
Y. Suzuki, Visiting Assoc, DB	R. Eastman, DDEMD, NIDDK
M. Taouis, Visiting Fellow, DB	M. Cool, Biologist, DB

COOPERATING UNITS *(if any)*
T. Kadowaki and H. Kadowaki, Tokyo, Japan; HW Rudiger and M. Dreyer, Hamburg, Germany; Li Al-Gazali, United Arab Emirates; L. Sinclair, London; G. Sesti; R. Lauro, Italy; C. Cochran, CC; Y. Zick, Israel; A. Dunaif, Hershey, PA;

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action

INSTITUTE AND LOCATION
NIDDK

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	4.1	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin resistance contributes to the pathogenesis of several disease states including obesity and noninsulin-dependent diabetes mellitus (NIDDM). We have investigated the nature of the genetic factors that predipose to the development of insulin resistance. Mutations have been identified in the insulin receptor gene in patients with several genetic syndromes associated with insulin resistance. Some mutations cause insulin resistance by decreasing the number of receptors on the cell surface by one of several mechanisms: impairing receptor biosynthesis; impairing the transport of receptors to the plasma membrane; or accelerating the rate of receptor degradation. Other mutations cause insulin resistance by impairing one or more functions of the insulin receptor: decreasing the affinity of insulin binding; or impairing receptor tyrosine kinase activity. Recently, we have developed an animal model of this disease: a mouse in which the insulin receptor gene has been inactivated by homologous recombination. In order to investigate the significance of heterozygosity for a mutation in the insulin receptor gene, we have investigated the effect of mutations in the context of hybrid receptors formed by dimerization of a mutant half-receptor with a wild type half-receptor. In the case of the Leu-323 mutation (a mutation in the extracellular domain that inhibits insulin binding), the hybrid receptor bound insulin with normal affinity. This explains the observation that the Leu-323 mutation causes insulin resistance in a recessive fashion. In contrast, mutations in the intracellular domain (e.g., Ile-1153 mutation) generally inhibit receptor tyrosine kinase activity, and cause insulin resistance in a dominant fashion. However, Ile-1153 mutant receptors do not appear to dimerize with wild type receptors when both cDNAs are expressed in the same cells. The dominant effect of the Ile-1153 mutation may be explained by competition for binding of downstream effector molecules. While the prevalence of mutations in the insulin receptor gene is not known with certainty, most patients with NIDDM appear not to have mutations in that gene. Thus, studies have been initiated to screen for the presence of mutations in the gene encoding insulin receptor substrate-1 (IRS-1). Several variant sequences have been identified in the IRS-1 gene, and the prevalence of these variant sequences appears to be increased in patients with NIDDM. Studies are underway to determine whether these variant sequences impair the function of IRS-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 47024-16 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthetic labeling of the insulin receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P. Gorden	Director	NIDDK
Others:	S.I. Taylor	Chief	DB/NIDDK

COOPERATING UNITS *(if any)*

J-L Carpentier, Geneva, Switzerland
L. Beitz, Howard Hughes, Bethesda; E. Collier, CIB/NIAID

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
    ☐ (a1) Minors
    ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DISCONTINUE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 47026-11 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin receptors and molecular mechanisms of insulin action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S.I. Taylor	Chief	DB< NIDDK	D. Accili	Vis. Sci	DB
Others:	R. L.-Toledano	Vis. Sci	DB, NIDDK	M. Quon	Clin.Assoc	DB
	M. Taouis	Vis. Fel	DB, NIDDK	MJ Zarnowski	Biologist	DB
	P. Gorden	Director	NIDDK	S. Cushman	Sec.Chief	DB
	N. Philippe	Bio.Aide	DB, NIDDK	B. Ing	Pre-IRTA	DB
	Y. Suzuki	Vis.Ass.	DB, NIDDK	E.Wertheimer	Vis.Fel.	DB
	P. Roach	Clin.Ass.	DB, NIDDK	H. Chen	Vis. Assoc.	DB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.2	6.1	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the first step in insulin action, insulin binds to its receptor on the surface of the target cell, thereby activating the insulin receptor tyrosine kinase. This leads to receptor autophosphorylation, and to phosphorylation of tyrosine residues in multiple intracellular proteins. We have investigated this pathway in isolated rat adipocytes, a physiologically important target cell for insulin action. When recombinant epitope tagged GLUT4 glucose transporters are expressed in adipocytes, insulin stimulates the recruitment of these glucose transporters to the cell surface. When wild type human insulin receptors are co-expressed in rat adipocytes, this leads to a marked increase in the number of epitope tagged glucose transporters on the cell surface. The insulin receptor must possess intact tyrosine kinase activity in order to stimulate glucose transport in rat adipocytes. Thus, it is of interest to determine which intracellular proteins function downstream from the receptor tyrosine kinase in the pathway that leads to translocation of GLUT4 molecules to the cell surface. Insulin receptor substrate-1 (IRS-1) is among the best characterized substrates for phosphorylation by the insulin receptor. When IRS-1 levels were decreased by expression of an anti-IRS-1 ribozyme (designed to cleave rat IRS-1 mRNA), this impaired the ability of insulin to stimulate the translocation of epitope-tagged GLUT4 to the cell surface in isolated rat adipocytes. Furthermore, expression of human IRS-1 in rat adipocytes increased the number of epitope-tagged GLUT 4 molecules on the cell surface These observations suggest that IRS-1 mediates (at least in part) the effect of insulin to stimulate glucose transport. IRS-1 undergoes multi-site phosphorylation, thereby activating multiple SH2-domain-containing proteins that function in the insulin action pathway. Because expression of a dominant negative mutant of phosphatidyl inositol 3-kinase (PI-3-K) inhibits insulin action in rat adipocytes, this suggests that PI-3-K functions downstream from IRS-1. In contrast, dominant negative mutant of ras does not inhibit the ability of insulin to stimulate translocation of epitope-tagged GLUT4. For example, we have identified a membrane glycoprotein (pp120/HA4) in rat liver that undergoes insulin-stimulated tyrosine phosphorylation. By expressing recombinant rat pp120/HA4 in cultured NIH-3T3 cells, we confirmed that the insulin receptor phosphorylates tyrosine residues in the intracellular domain of pp120/HA4. Moreover, in NIH-3T3 cells that co-express recombinant human insulin receptors and rat pp120/HA4, insulin acquires the ability to activate ecto-ATPase activity. The physiological role of pp120/HA4 is currently under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 47027 - 10 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Somatostatin Analog (SMS 201 - 995) in hormone secreting tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	R.C. Eastman	Director	DDEMD, NIDDK
OTHERS:	C. M. Hendricks	Bio Lab Tech.	DB, NIDDK
	P. Gorden	Director	NIDDK
	P. Roach	Clinical Assoc.	DB, NIDDK

COOPERATING UNITS *(if any)*
B. Weintraub Chief, MCNEB, NIDDK

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a project to study the long-term effects of the treatment of acromegaly with the long-acting somatostatin analog, octreotide. Patients are treated in an unblinded fashion with octreotide as long as it is medically necessary. Treatment and prevention of gallbladder sludge and gallstones, common side effects of long-term octreotide therapy, is being attempted with ursodeoxycholate, a bile salt which has been used to dissolve gallstones in the general population. Patients who are candidates for treatment with octreotide, and who have normal gallbladders as determined by ultrasonography, are randomized to receive either ursodeoxycholate or placebo in order to study whether ursodeoxycholate is effective in preventing octreotide-induced gallbladder sludge or stones are treated in an unblinded fashion with ursodeoxycholate in an attempt to dissolve the sludge or stones. Fifteen patients have been randomized to masked treatment with ursodeoxycholate, and nine patients have been treated unmasked for sludge or stones. To date, pre-existing gallstones have dissolved in 3 of 5 patients and pre-existing sludge has disappeared in 2 of 4 patients during ongoing therapy with octreotide. Octreotide has been approved by the FDA for use in acromegaly. Thus, enrollment has ceased in this protocol and the protocol will be converted to a natural history study in the next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 47028-06 DB

PERIOD COVERED

October 1, 1994 to September 31, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of genes encoding proteins that mediate insulin action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S.I. Taylor	Chief,	DB, NIDDK
	H. Chen	Spec. Vol,	DB, NIDDK
	S. Najjar	IRTA	DB, NIDDK
	D. Accili	Vis. Sci.	DB, NIDDK
	N. Phillipe	Stu.Sup.IRTA	DB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47029 - 04 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mathematical modeling of glucose metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. Quon	Clinical Associate	DB/NIDDK
Others:	R. Eastman	Director	DDEMD/NIDDK
	S.I. Taylor	Chief	DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Diabetes Branch

SECTION

Receptors & Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin resistance occurs in a variety of pathological states including obesity, non-insulin dependent diabetes mellitus (NIDDM) and hypertension. Decreased glucose effectiveness has been reported in NIDDM. Since insulin resistance and impaired glucose effectiveness may play a role in the pathogenesis of NIDDM, quantitative assessment of these properties is of interest. Recently, a minimal model approach involving mathematical modeling and computer simulation has been used to estimate both insulin sensitivity and glucose effectiveness from the results of a single frequently sample intravenous glucose tolerance test (FSIVGTT). The equations of the minimal model describe changes in plasma glucose concentration as functions of insulin and glucose concentrations. A computer program identifies model parameters that generate a best fit to insulin and glucose data obtained during the FSIVGTT. Thus, the minimal model is able to estimate the relative contributions of insulin and glucose to glucose tolerance. We have used the minimal model to generate specific predictions of both insulin-dependent and insulin-independent glucose metabolism under a variety of conditions. We performed experiments in subjects with insulin dependent diabetes mellitus and in normal subjects to test these predictions. By comparing model predictions with experimental results, we were able to demonstrate that the minimal model underestimates the contributions of insulin and overestimates the contribution of glucose to glucose metabolism. Studies are presently underway to confirm these results and to understand the origin of the discordance between minimal model predictions and experimental results. The data that we collect may allow us to formulate a more accurate and useful mathematical model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47030-04 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin Receptor-Related Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Simeon I. Taylor	Chief, DB,	DB, NIDDK
Others:	Hsiang Yiang Jui	Spec. Vol,	DB, NIDDK
	Yoshifumi Suzuki	Vis. Assoc.	DB, NIDDK
	Domenico Accili	Vis. Sci	DB, NIDDK

COOPERATING UNITS *(if any)*

Paul Goldsmith, NIDDK; Joseph Schlessinger, New York University

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The insulin receptor is encoded by a single copy gene located on chromosome 19 in the human. In addition, at least two other highly homologous genes have been identified. One gene encodes the type 1 insulin-like growth factor (IGF) receptor. The third gene in the family encodes an orphan receptor tyrosine kinase that has been named the insulin receptor-related receptor (IRR). We have cloned the cDNA molecules encoding human and mouse IRR. Generally, the predicted structure of IRR is similar to those of the receptors for insulin and IGF-1. In the case of human IRR, we detected a splicing variant in which 36 nucleotides from the 3'-end of intron 13 is included between the sequences of exons 13 and 14. Two observations suggest that this alternatively spliced form may not be physiologically important. First, this variant species of IRR mRNA represents <10% of IRR mRNA in adult human kidney. Second, this splicing variant is not evolutionarily conserved; although there is a potential alternate splice acceptor site 35 nucleotides from the end of intron 13 in mouse IRR, this alternate splicing pattern would be predicted to shift the reading frame. Several variant splicing patterns were observed in the region of the junction between exons 1 and 2 of mouse IRR. None of these variant species of mouse IRR mRNA conserved the open reading frame. Inasmuch as they are not predicted to encode functional IRR molecules, it is unlikely that they are physiologically significant. When IRR cDNA is expressed by transfection in NIH-3T3 cells, the IRR precursor undergoes post-translational processing steps including N-linked glycosylation and proteolytic cleavage into distinct α- and β-subunits. When cells expressing recombinant IRR are incubated with vanadate (an inhibitor of phosphotyrosine phosphatases), this leads to an increase in the phosphotyrosine content of IRR. However, the phosphotyrosine content of IRR is not increased by incubating the cells with insulin, IGF-1, or proinsulin. Further investigations are underway to attempt to identify the natural ligand for IRR, and to determine the physiological role of IRR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47031-04 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. Reitman	Senior Staff Fellow	DB, NIDDK
Others:	Oksana Gavrilova	Visiting Fellow	DB, NIDDK
	Yufang He	IRTA	DB, NIDDK
	Mark Mason	IRTA	DB, NIDDK

COOPERATING UNITS *(if any)*

Heiner Westphal and Eric Lee, LMG, NICHD; Joseph Grasso, University of Connecticut Health Center; Michael J. Quon, HEB, NHLBI.

LAB/BRANCH

Diabetes Branch

SECTION

Molecuar Biology and Gene Regulation

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal is to understand how multi-gene clusters are organized and regulated. Specifically, how does the physical organization influence expression of the genes and how is a particular gene within an open cluster chosen for expression? The model evolving from studies of the human β-globin cluster is that control elements upstream of the genes are essential for locus activation (chromatin opening). These upstream elements also increase the expression of the nearest available genes, with availability chiefly determined by the promoter. This model is supported by the genotype/phenotype correlations observed in patients with deletional β-thalassemia and hereditary persistence of fetal hemoglobin.

We have shown previously that the chicken β-globin gene, with its 3' enhancer, is expressed in a copy number dependent manner in transgenic mice. The expression level increased ~6-fold upon inclusion of the four upstream DNase hypersensitive sites. Each of the four β-like globins were shown to be under the control of both the upstream hypersensitive sites and the enhancer located between the β and ε genes. To study the effect of the individual upstream hypersensitive sites, we produced lines of mice carrying a single site linked to the β gene and enhancer. These experiments demonstrated that no single upstream hypersensitive site accounts for the increased β-globin expression seen in mice containing the complete upstream region. Taken together, our work demonstrates that at least three regions (the β/ε enhancer and at least two upstream sites) plus the promoter are required for proper expression of the individual β-like globins. Butyrate stimulates the expression of fetal globins in adult cells and is undergoing clinical trials for this purpose. We used transient expression to explore the mechanism of butyrate's actions and found two effects. First, low concentrations of butyrate caused an inhibition of reporter expression. The second effect was stimulation of reporter expression and required higher butyrate concentrations and was promoter-dependent. We postulate that the initial inhibition is due to butyrate-influenced packaging of the transfected DNA into a less transcriptionally active configuration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 47032- 01 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular genetics and pathophysiology of obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Simeon Taylor	Chief	DB, NIDDK
Others:	Valarie Barr	IRTA	DB, NIDDK
	Cassandra Constantino	Vis. Assoc.	DB, NIDDK
	Marc Reitman	SSF	DB, NIDDK

COOPERATING UNITS *(if any)*

Paul Goldsmith, NIDDK; Allen Spiegel, NIDDK

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is considerable evidence suggesting that genetic factors predispose to the development of obesity. Recent work from several laboratories has begun to elucidate the molecular basis of several forms of genetic obesity in rodents. We have initiated a project to extend the work emanating from the positional cloning of the murine obese gene. The gene encodes a peptide hormone (i.e., leptin) that is secreted by adipose tissue. We have used this knowledge to develop expression vectors for synthesis of recombinant murine and human leptin. Using recombinant leptin, we have initiated efforts to search for a cell surface receptor that mediates the action of leptin upon target cells. In addition, we have obtained antibodies to leptin, and are attempting to establish a radioimmunoassay to measure leptin levels in plasma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47033- 01 DB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genes ancestral to the thyroid/steroid receptor family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J. Edward Rall	Senior Scientist	DB, NIDDK
Others:	Z. Kostrouch	Visiting Associate	DB, NIDDK
	M. Kostrouchova	Special Volunteer	DB, NIDDK

COOPERATING UNITS *(if any)*

Joram Piatigorsky, LMDB, NEI; Mike Krause, LMB, NIDDK; Alan Kimmel, LCDB, NIDDK.

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our group has been investigating members of thyroid/steroid hormone receptor family in lower organisms in an attempt to analyze the structure and functions of the most primitive receptors, better to understand the function of the myriad of receptors present in the vertebrata. We cloned cDNAs for three such proteins in the nematode Caenorhabditis elegans. One of them (CHR3) is highly homologous to the DHR3 in Drosophila and MHR3 in Manduca sexta. This receptor in Drosophila is induced by ecdysone. Another receptor we cloned (CNR14) is homologous to E78A receptor, also ecdysone inducible in Drosophila. The third receptor, CNR8 is similar to an early response gene in vertebrates, NAK1. The receptors are stage specific as shown by Northern analysis. More recently, we have cloned the genes for CHR3 and CNR14 and we are studying their promoters. We found that two genes, CHR3 and CNR14 are trans-spliced and so are organized in operons. While CHR3 is trans-spliced with a leader sequence SL2, and CNR14 is trans-spliced with SL1, CNR8 seems to be transcribed as single transcript. Cistronic transcription gives us the opportunity to study the genes which are transcribed from a common promoter and which could function together. We mapped CHR3 to the center region of chromosome 1 in close relation to hlh-2 gene, a homeobox protein analogous to E12 protein, the partner of MyoD in vertebrates. We confirmed the propinquity of the hlh-2 and CHR3 by PCR on a cosmid. Interestingly, some known mutants with deletion in this region are lethal. We are currently studying expression of CHR3 and CNR14 using microinjetions to the ovary of hermaphrodites with probable promoter regions coupled to DNA sequence coding for beta-galactosidase. We also hope to use heterozygotic strains with deletions in a region where CHR3 maps for a study of knock-out and rescue. We continued on the steroid hormone receptor in cubomedusan jellyfish Tripedalia cystophora. We cloned at least two cDNA isoforms and most of the gene. As far as we can tell, this is the only such gene in his organism and could represent the most primitive member of thyroid/retinoic acid subfamily.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 48001-03 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin Cell Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Ian A. Simpson, Visiting Scientist, DB/NIDDK
Others: Samuel W. Cushman, Chief, EDMNS, DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 DK 48002-04 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin's Regulation of Glucose Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)*
PI: Samuel W. Cushman Chief, EDMNS DB/NIDDK
Others: I.A. Simpson, Visiting Scientist, DB/NIDDK; C.M. Ferrara, IRTA, DB/NIDDK; K.I. Timmers, Special Volunteer, DB/NIDDK; C.M. Wilson, IRTA, DB/NIDDK; D.A. Malide, Visiting Fellow, DB/NIDDK; T. Ploug, Special Volunteer, DB/NIDDK; J.T. Brozinick, Special Volunteer, DB/NIDDK; C.S. Hinck, Special Volunteer, DB/NIDDK; J.-F. St.-Denis, Visiting Fellow, DB/NIDDK; T.H. Reynolds, preIRTA, DB/NIDDK; D.R. Yver, Chemist, DB/NIDDK; M.J. Zarnowski, Biologist, DB/NIDDK; M.J. Quon, Clinical Associate, DB/NIDDK.

COOPERATING UNITS *(if any)*
Mount Sinai School of Medicine, Shizuoka General Hospital, The University of Bath, The Hospital for Sick Children (Toronto), Emory University School of Medicine, Stanford University School of Medicine, Palo Alto Medical Foundation, Baylor College of Medicine, Harvard Medical School, Tufts-New England Medical Center.

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION

TOTAL STAFF YEARS: 9.9	**PROFESSIONAL:** 9.9	**OTHER:** 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Staurosporine, a widely used protein kinase C inhibitor, completely inhibits both PMA- and insulin-stimulated glucose transport activity in isolated rat adipocytes, possibly at a step that regulates the specific translocation of the glucose transporters to the plasma membrane. Human adipose cells are much less responsive to insulin stimulation of glucose transport activity than are rat adipocytes. The intracellular retention process appears to have adapted to release, in the basal state, a greater proportion of the total-cellular pool of GLUT4 to the cell surface of the larger human adipocytes. As a consequence of this adaptation to cell size and surface area, the residual intracellular-reserve pool of GLUT4 that is available to respond to insulin is lower in the human than in the rat adipocyte. We have used a photoaffinity label to quantitate the glucose transporters on the surface of BC3H-1 cells. This technique demonstrates that glucose prefeeding 1) decreases the total number of GLUT1 and 2) redistributes the majority of the remaining transporters to an intracellular site where they can now be translocated to the cell surface in response to insulin and PMA. The effects of insulin on the cell surface quantities of GLUT1, GLUT3, and GLUT4 glucose transporters in L6 myotubes were determined. Insulin stimulated the translocation of all three glucose transporter isoforms to the plasma membrane fraction from the light microsome fraction, resulting in equal molar quantities on the cell surface. To test the hypothesis the T_3 increases the partitioning of GLUT1 to the cell surface in the liver-derived ARL 15 cell line, we quantitated cell surface GLUT1. We conclude that most of the early (6 h) stimulation of glucose transport by T_3 in ARL 15 cells is mediated by an increase in the partitioning of GLUT1 to the plasma membrane. With more chronic T_3 treatment (48 h), the enhanced surface partitioning of GLUT1 is persistent, and is superimposed on an increase in total cellular GLUT1, accounting for a further increase in glucose transport. We have observed that LDL receptors localized in intracellular compartments of isolated rat adipose cells seem to be functionally regulated since insulin acutely diminishes the number of receptors by accelerating their rate of degradation. We tested the hypothesis that sulfonylureas promote glucose uptake into 3T3-L1 cells or isolated rat adipocytes through specific high affinity receptors. We conclude that the anti-diabetogenic effects of sulfonylureas are not mediated by a direct action of sulfonylureas to increase glucose uptake into adipose tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48003-04 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Samuel W. Cushman, Chief, EDMNS, DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20893

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48005-04 DB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Counterregulation of Insulin's Action by Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Ian A. Simpson, Ph.D.	Visiting Scientist	EDMNS/DB/NIDDK
Others:	Samuel W. Cushman, Ph.D.	Chief	EDMNS/DB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 48007-03 DB

PERIOD COVERED
October 1, 1994 to September 31, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Glucose Transport in Mammalian Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)*

Ian A. Simpson, Ph.D.	Visiting Scientist	EDMNS/DB/NIDDK
Frances Maher, Ph.D.	Visiting Fellow	EDMNS/DB/NIDDK
Lynn R. Sorbara, Ph.D.	Staff Fellow	EDMNS/DB/NIDDK
Theresa M. Davies-Hill	Biologist	EDMNS/DB/NIDDK
Kang Li, M.D., Ph.D.	Special Volunteer	EDMNS/DB/NIDDK
Ellen M. Koehler, Ph.D.	Special Volunteer	EDMNS/DB/NIDDK

COOPERATING UNITS *(if any)*
Dr. P. Davies/Albert Einstein College of Medicine, Dr. P. Lysko/SmithKline Beecham, Dr. K. Seamon/CBER/DBB/FDA, Dr. C. Smith/NIMH/NIH, Drs. W. Tresher and J. Latterra/Johns Hopkins, Drs. E. Novotny and R. Sherwin/Yale U., Dr. M. Levine/NIDDK/NIH, Drs. N. Appel and Q. Smith/NIA/NIH, Dr. S. Vannucci/Hershey

LAB/BRANCH
Diabetes Branch

SECTION
Experimental Diabetes, Metabolism, and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bldg. 10, Rm. 5N102, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glucose is the primary energy source for mammalian brain and its uptake across the blood-brain barrier (BBB) and into the neurons and glia that make up the brain is mediated by a family of glucose transporters (GLUTs). The 55kDa isoform of GLUT1 facilitates the transcytosis of glucose across the endothelial cells that constitute the BBB. It has been proposed that this transporter is asymmetrically distributed between the luminal and abluminal membranes. The luminal concentration of GLUT1 was quantitated in control and diabetic animals by perfusion and subsequent photolysis of an impermeant photolabel $[^3H]$ ATB-BMPA. The ratio of luminal : total transporter was reduced in the microvessels of diabetic animals suggesting a mechanism of redistribution may account for the observed reduction of glucose flux. GLUT3 is the primary neuronal transporter and in cultured cerebellar granule neurons it exhibits both a high affinity ($K_m \approx 2.8$ mM for 3-O-methylglucose) and the highest transport rate (turnover number = 853 sec^{-1}) of all the GLUT isoforms measured. Thus the kinetic capacity of GLUT3 provides the neuron a survival advantage under conditions of metabolic stress such as hypoxia or hypoglycemia. Glucose consumption is enhanced when cultured hippocampal neurons are exposed to nitric oxide (NO), however, GLUT1 and GLUT3 expression is suppressed. bFGF and EGF both protect against NO toxicity and restore GLUT1 and GLUT3 expression to normoxic levels suggesting a relationship between fuel supply and neuronal survival. *In vivo* studies of hypoxia-ischemia in the 7 day old neonatal rat have revealed that dramatic increases in the levels of GLUT1 mRNA and protein occur very rapidly following the insult and persist in the ischemic hemisphere at 24h of recovery. Over a period of 24-48 h GLUT3 mRNA levels decline in a manner consistent with neuronal necrosis. In contrast, GLUT5 expression in microglia is unchanged at 24h but by 48h a dramatic increase is observed in the area of the infarct. Similar observations have now been made in an adult rat model of focal ischemia. In a clinical setting, a small group of children have recently been identified with a deficiency in GLUT1 glucose transporter which results in an impaired ability to transport glucose across the BBB. This defect is manifested by infantile-onset seizures and persistent hypoglycorrachia. A screening system has been devised to identify other children with defects in either GLUT1 or GLUT3 transporters which might induce seizure activity. Such children have been found to respond positively to the Ketogenic Diet which effectively provides ketone bodies as an alternative energy source to glucose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 51000-37 CHB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Immunology of Blood Cell Deficiencies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Raphael Shulman	Chief	CHB:NIDDK
Others:	Diane M. Reid	Expert	CHB:NIDDK
	Ruyi He	Visiting Associate	CHB:NIDDK
	Charles E. Jones	Chemist	CHB:NIDDK

COOPERATING UNITS (if any)

Monica Yu (CC-DTM); Charles Carter (CC-DTM)
R. Cohen (Genentech)

LAB/BRANCH Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Idiopathic thrombocytopenic purpura, the most common acquired hemorrhagic disorder, is characterized by platelet destruction due to antiplatelet autoantibodies. Treatment is empirical, based on general measures of immunosuppression or reticuloendothelial blockade. We have sought to determine the basis for the disorder, establish diagnostic and prognostic serologic tests, and develop innovative therapy.

Using strategies of cloning cDNA fragments of a major platelet membrane glycoprotein target of ITP antibodies (GPIb); expressing the respective protein fragments; synthesizing peptides of high antigenic indices within reactive fragments; and epitope scanning with overlapping 15-mer peptides, we have localized a chief epitope on GPIb to the sequence TKEQTTFPP. This sequence has partial homology to 15 known proteins, but none appear to be reasonable cross-reactive immunogens. Soluble GP peptides or protein fragments we studied were not effective competitors for ITP Abs that attach to intact platelets, but may be valuable solid-phase adsorbents in therapeutic pheresis procedures.

Applications of flow cytometry, antigen capture radioimmunoassays, and newly developed electrochemiluminescence immunoassays to the detection of plasma ITP Abs indicate the Abs are too low in affinity or concentration to be measured effectively for clinical utility. In contrast, antibody eluates of patients' platelets or concentrated indirect eluates prepared with their plasma are reliable sources of potent Ab for diagnostic and possibly prognostic applications.

The recently discovered stimulator of megakaryocyte proliferation and maturation, thrombopoietin (TPO), was found, in collaboration with R. Cohen at Genentech, who developed an ELISA system for this ligand, to be below normal levels in sera of 18 ITP patients with severe thrombocytopenia. This indicates that TPO may be regulated by megakaryocyte mass rather than platelet number and that it should be considered for trials as a therapy for ITP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 51001-37 CHB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Raphael Shulman	Chief	CHB:NIDDK
Others:	Diane M. Reid	Expert	CHB:NIDDK
	Charles E. Jones	Chemist	CHB:NIDDK
	Kazuhiko Matsuo	Special Volunteer	CHB:NIDDK

COOPERATING UNITS (if any)

Junichi Honda, Kurume University, Japan
Ben Wolozin, LCS:NIMH

LAB/BRANCH Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.5

PROFESSIONAL: 1.0

OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Platelets are prototypic adhesive cells that have several major membrane glycoproteins which bind a variety of plasma and subendothelial molecules involved in maintaining vascular integrity and initiating hemostasis or thrombosis.

We have continued studies of a rare hemorrhagic disorder, thrombasthenia, in which platelets have deficient or defective fibrinogen-binding and aggregation-promoting glycoprotein, GPIIb/IIIa. We found one patient had markedly reduced GPIIb mRNA and variant splicing with a partial deletion of exon 28, that proved on population studies to be an unusual alternative of a normal polymorphism. There were no sequence defects in the putative promoter/enhancer regions nor previously reported point mutations. Complete genomic DNA for GPIIb is currently being sequenced for insight into abnormalities of this major integrin, unique to platelets, which is pivotal in modulating platelet function.

Alzheimer's disease is associated with brain deposition of plaques of a peptide called beta-amyloid. Beta-amyloid is normally present in plasma at levels of 225-625 pM. There is no function attributed to the soluble peptide. We found that soluble beta-amyloid at concentrations found in plasma augmented ADP-induced platelet aggregation 2-fold. The activation process was blocked by RGD inhibition of GPIIb/IIIa fibrinogen-binding sites. Partial beta-amyloid sequence (amino acids 1-16) was weakly active; the reverse sequence (40-1) was inactive. Beta-amyloid alone induced tyrosine phosphorylation of a 180 kD platelet protein, suggesting that physiologic plasma levels of this peptide may act through a specific tyrosine kinase-linked receptor.

Hemorrhagic disorders due to alloantibodies against polymorphic antigens on platelet membrane GPs occur after transfusions and in neonates of sensitized mothers. Diagnosis and prophylaxis of these disorders depend on accurate pheno-or geno-typing. We develped restriction enzyme assays to allotype two different platelet antigen groups, Yuk and Br, using genomic DNA as a time-saving alternative to existing methods requiring platelet cDNA and Southern blotting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52008-16 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression and Human Genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.D. Camerini-Otero	Chief	GBB, NIDDK	
Others:	E. Angov	IRTA Fellow	J. Yancey-Wrona	IRTA Fellow
	L. Ferrin	Research Associate	G. Poy	Biologist
	Hai Kinal	IRTA Fellow	Oleg Voloshin	Visiting Associate
	Lijiang Wang	IRTA Fellow	Anita Szwast	pre-IRTA
	Vlad Malkov	Visiting Fellow		

COOPERATING UNITS (if any)
Carol Camerini-Otero - Diabetes Branch, NIDDK
Victor Zhurkin and Robert Jernigan - Laboratory of Mathematical Biology, NCI
Asad Sheikh - Division of Intramural Research, NCHGR

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
10.00	9.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to dissect the biochemical steps involved in genetic recombination we have chosen to focus on a key early step: strand exchange between homologous single-stranded and duplex parental DNAs. We have established a new paradigm for this homologous pairing. In essence, that recombinases such as the *E. coli* RecA protein can form a novel DNA triplex (R-form DNA) in which the third strand may have any arbitrary sequence and must have a parallel orientation with respect to the phosphodiester backbone of the identical strand in the duplex. We have also been able to isolate synaptic complexes consisting of all the three strands and RecA. In order to understand the mechanism and structures involved in greater detail we have determined that a 20 amino acid peptide from RecA has some of the activities of RecA. Most remarkably, this short 20 amino acid peptide spanning the disordered or mobile loop L2 of RecA is capable of carrying out the hallmark reactions mediated by the whole RecA protein: pairing (trageting) of a single-stranded DNA to its homologous site on duplex DNA and forming homology-dependent stable joint molecules. Inthe course of the reaction the peptide binds to both substrates, unstacks the single-stranded DNA and assumes a β-structure. Efforts are now underway to determine the three-dimensional structure of this peptide when bound to DNA. The synaptic complexes have also been used to develop a method for the selective cleavage of human DNA (RecA-Assisted Restriction Endonuclease (RARE) cleavage). We have shown that RARE can be used to map sequences in genomic DNA of increasing complexity, including human DNA. Recently, we have developed a new technique, RecA-Assisted Cloning (RAC), that is functionally the reverse of RARE in that uses RecA and oligonucoetides to direct sequence-specific ligation of genomic DNA.

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52012-11 GBB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Relationships of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard L. Proia	Research Biologist	GBB, NIDDK
Others:	Debra Boles	IRTA	GBB, NIDDK
	Olivia N. Johnson	Summer IRTA	GBB, NIDDK
	Francine Norflus	Biologist	GBB, NIDDK
	Kazunori Sango	Visiting Fellow	GBB, NIDDK
	Mark Pennybacker	IRTA	GBB, NIDDK
	Yujing Liu	IRTA	GBB, NIDDK
	Yasuharu Okuda	pre-IRTA	GBB, NIDDK

COOPERATING UNITS (if any)

Jacqueline N. Crawley, NIMH, NIH; Konrad Sandhoff, Institut für Organische Chemie und Biochemie der Universität Bonn, Bonn, Germany; Kinuko Suzuki, Department of Pathology, Univ. North Carolina at Chapel Hill; Heiner Westphal, NICHD, NIH

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
5.45	5.25	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tay-Sachs and Sandhoff diseases are severe neurodegenerative disorders characterized by impaired G_{M2} ganglioside degradation as a consequence of β-hexosaminidase A deficiency. Mutations in the *HEXA* and *HEXB* genes, which encode the subunits of β-hexosaminidase A , cause Tay-Sachs and Sandhoff disease, respectively. In humans, the neurologic phenotype and neuropathology in the two diseases are very similar.

Through disruption of the *Hexa* and *Hexb* genes in embryonic stem cells, we have established mouse models corresponding to Tay-Sachs and Sandhoff diseases. Mice homozygous for either the disrupted *Hexa* or *Hexb* gene were deficient in β-hexosaminidase A and accumulated G_{M2} in their brains. However, unlike Tay-Sachs and Sandhoff disease patients, the *Hexa* and *Hexb* "knock-out" mice displayed very different phenotypes. The *Hexa* knock-out mice had a normal life span (~2 years) and showed no neurologic abnormalities. In contrast, the *Hexb* knock-out mice were severely affected. Beginning at about 3.5 months these animals showed progressive gait abnormalities, muscle wasting and spasticity. By 5 months the animals displayed nearly complete limb paralysis. Consistent with the different phenotypes were the number and distribution of storage neurons in the two types of mutant mice. In the *Hexb* knock-out mice storage was observed in almost all neurons in the central nervous system. In contrast, in the *Hexa* knock-out mice storage was restricted to certain regions of the brain. In light of the phenotypic difference, it is notable that significant storage was found in cerebellar Purkinje cells and in the spinal cord motor neurons in the *Hexb* but not the *Hexa* knock-out mice. Furthermore, we have found that phenotypic difference between the two mouse models is the result of differences in the ganglioside degradation pathway between mice and humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52015-06 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Studies of Protein-DNA Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peggy Hsieh	Sr. Staff Fellow	GBB, NIDDK
Others:	Indranil Biswas	Visiting Fellow	GBB, NIDDK
	Mikhail Grigoriev	Visiting Fellow	GBB, NIDDK
	Akira Yamamoto	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We seek to understand at a molecular level the various ways by which an organism maintains the integrity of its genome while accommodating the need for diversity. Our research efforts currently focus on two related processes, homologous recombination and DNA mismatch repair.

We are characterizing a key step in genetic recombination, DNA branch migration. Pairing of two homologous DNA duplexes and strand exchange carried out by recombination proteins like *E. coli* RecA protein lead to the formation of a central intermediate in recombination, the Holliday junction. The Holliday junction, corresponding to the exchange point between two homologous DNAs, can migrate by the step-wise breakage and reformation of hydrogen bonds between identical DNA strands in two duplexes. This process is known as branch migration and it dictates the amount of genetic information transferred from one chromosome to another. Recent efforts have focused on the pivotal role of the structure of the Holliday junction in determining the rate of spontaneous DNA branch migration. Conformational changes induced in the Holliday junction by lowering the concentration of magnesium ions lead to a rapid acceleration in the rate of branch migration. Our findings indicate that adoption of a Holliday junction conformation in which base stacking is disrupted at the crossover point removes a kinetic barrier to branch migration.

Mismatch repair, exemplified by the *E. coli* methyl-directed mismatch repair pathway, plays critical roles in the preservation of the integrity of a genome. Mismatches can arise through DNA replication errors, homologous recombination and spontaneous DNA damage. Components of the bacterial mismatch repair system encoded by the *mutS* and *mutL* genes in *E. coli*, are highly conserved in both prokayotes and eukaryotes and act to regulate levels of homologous recombination. Recently, defects in human genes encoding mismatch repair enzymes have been implicated in hereditary colon cancer. We are interested in understanding the molecular mechanism involved in mismatch recognition by the MutS protein. To this end, we have cloned a gene encoding a thermostable MutS protein from *Thermus aquaticus*. Initial characterization of the *T. aquaticus* MutS protein demonstrates that it has thermostable DNA mismatch binding and ATPase activities.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52015-06 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Molecular Studies of Protein-DNA Interactions

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Peggy Hsieh	Sr. Staff Fellow	GBB, NIDDK
Others:	Indranil Biswas	Visiting Fellow	GBB, NIDDK
	Mikhail Grigoriev	Visiting Fellow	GBB, NIDDK
	Akira Yamamoto	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We seek to understand at a molecular level the various ways by which an organism maintains the integrity of its genome while accommodating the need for diversity. Our research efforts currently focus on two related processes, homologous recombination and DNA mismatch repair.

We are characterizing a key step in genetic recombination, DNA branch migration. Pairing of two homologous DNA duplexes and strand exchange carried out by recombination proteins like *E. coli* RecA protein lead to the formation of a central intermediate in recombination, the Holliday junction. The Holliday junction, corresponding to the exchange point between two homologous DNAs, can migrate by the step-wise breakage and reformation of hydrogen bonds between identical DNA strands in two duplexes. This process is known as branch migration and it dictates the amount of genetic information transferred from one chromosome to another. Recent efforts have focused on the pivotal role of the structure of the Holliday junction in determining the rate of spontaneous DNA branch migration. Conformational changes induced in the Holliday junction by lowering the concentration of magnesium ions lead to a rapid acceleration in the rate of branch migration. Our findings indicate that adoption of a Holliday junction conformation in which base stacking is disrupted at the crossover point removes a kinetic barrier to branch migration.

Mismatch repair, exemplified by the *E. coli* methyl-directed mismatch repair pathway, plays critical roles in the preservation of the integrity of a genome. Mismatches can arise through DNA replication errors, homologous recombination and spontaneous DNA damage. Components of the bacterial mismatch repair system encoded by the *mutS* and *mutL* genes in *E. coli*, are highly conserved in both prokayotes and eukaryotes and act to regulate levels of homologous recombination. Recently, defects in human genes encoding mismatch repair enzymes have been implicated in hereditary colon cancer. We are interested in understanding the molecular mechanism involved in mismatch recognition by the MutS protein. To this end, we have cloned a gene encoding a thermostable MutS protein from *Thermus aquaticus*. Initial characterization of the *T. aquaticus* MutS protein demonstrates that it has thermostable DNA mismatch binding and ATPase activities.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-854-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52016-03 GBB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Thyroid hormone interactions with cells and proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jacob Robbins	Scientist Emeritus	GBB, NIDDK
Others:	Hans Cahnmann	Scientist Emeritus	GBB, NIDDK
	Salvatore Benvenga	Courtesy Associate	GBB, NIDDK

COOPERATING UNITS (if any)

D. Rader, Division of Intramural Research, NHLBI; Y. Ito, Division of Intramural Research, NHLBI

LAB/BRANCH Genetics and Biochemistry Branch

SECTION Office of the Chief

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 2.7	PROFESSIONAL: 2.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies to define the physiological role of thyroid hormone binding to serum lipoproteins have continued. In the most recent experiments, the effect of various lipoproteins and other thyroxine-binding proteins of serum on the release of ^{131}I-labeled thyroxine from cultured human fibroblasts was examined. Although all the thyroxine-binding proteins appear to facilitate exit by acting as a sink for hormone released from the cells, preliminary results suggest that LDL has a differential effect on thyroxine release from normal fibroblasts and from fibroblasts lacking LDL receptors. At appropriate concentrations, LDL enhances release from the normal cells but not from the abnormal cells.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
01 DK 52017-03 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Thyroid Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Jacob Robbins, Scientist Emeritus, GBB, NIDDK
Others: J. Desiree Pineda, Special Volunteer, GBB, NIDDK

COOPERATING UNITS *(if any)*

J. Reynolds, Nuclear Medicine, CC; M. Merino, Laboratory of Pathology, NCI; C. Meyers, NCI; R. Alexander, Surgery Branch, NCI; D. Fraker, Surgery Branch, NCI; R. Jensen, University of California, San Francisco

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.0	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts have continued on methods to improve the management of high risk thyroid cancer. In one, low dose doxorubicin was used as a radiation enhancer and combined with I-131. Eight patients received 24 such treatments and are being evaluated for potential toxicity. No increased toxicity has been detected thus far.

A second method was to give I-131 therapy doses (150 to 300 mCi) to patients with negative diagnostic I-131 scans in whom the presence of cancer was demonstrated by thyroglobulin in blood. In 16 of 17 such patients, post-therapy scans were positive for recurrent or metastatic cancer. The treatment resulted in significant lowering of serum thyroglobulin levels and disappearance of I-131 uptake in lung metastases and other sites.

In collaboration with Dr. Ronald Jensen (Livermore National Laboratory and UCSF), the radiation-induced allelic loss of erythrocyte glycophorin A (MN blood type) was used as a biological dosimeter to quantitate radiation to bone marrow in thyroid cancer patients treated with I-131. The results showed an increase in NO and NN variant erythrocytes in proportion to the amount of bone marrow radiation calculated in the conventional manner. Since the effect is cumulative and persistent, it can be used in the evaluation of patients who have received I-131 therapy.

In collaboration with Bruce Weintraub, the development of methodology to utilize recombinant human TSH to stimulate I-131 uptake and thyroglobulin release in thyroid cancer is continuing. This will replace the current requirement for patients to become hypothyroid in order to test for residual or recurrent cancer. The multi-institutional Phase III data are now undergoing independent review prior to submission to the FDA for approval.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52019-03 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular biology of thyroid hormone receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Vera Nikodem	Chief, Mech Gene Reg Sect	GBB, NIDDK
Others:	Hideaki Tomura	Visiting Fellow	GBB, NIDDK
	Josef Lazar	Visiting Fellow	GBB, NIDDK
	Marcia Phyillaier	Biologist	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH: Genetics and Biochemistry Branch

SECTION: Mechanisms of Gene Regulation Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 3.7
PROFESSIONAL: 2
OTHER: 1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this study we have investigated the role of the amino terminal region of thyroid hormone receptors in thyroid hormone dependent transactivation of a thymidine kinase promoter containing thyroid hormone response elements composed either of a direct repeat or an inverted palindrome. Comparison of rat thyroid hormone receptor β1 with β2 provides an excellent model since they share identical sequences except for their amino termini. Our results show that thyroid hormone receptor β2 is an inefficient thyroid hormone dependent transcriptional activator. The degree of transactivation corresponds to that observed for the mutant which contains only those sequences common to thyroid hormone receptor β1 and β2. Using amino terminal mutants of thyroid hormone receptor β1 and transfection assays, we identified a region between amino acid 80 and 89 to be associated with thyroid hormone dependent transactivation function. Thyroid hormone receptor α1 amino-terminal mutants revealed that the transactivating property of thyroid hormone receptor α1 was localized between the 19 to 30 amino acids. Interestingly, we showed using a coimmunoprecepitation assay, that the differential interaction of the N-terminus of thyroid hormone receptor β1, β2 and α1 with transcription factor IIB correlates with the activation function. Hence, our results underscore the importance of the amino terminal region of thyroid hormone receptors in thyroid hormone dependent transactivation and suggest that a transactivating signal is transmitted to the general transcriptional machinery via a direct interaction of the receptor amino terminal region of thyroid hormone receptors with transcription factor IIB.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-854-809

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52020-03 GBB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of anteroposterior patterning in early frog development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sheryl Sato	Senior Staff Fellow	GBB, NIDDK
Others:	Song Huang	Visiting Fellow	GBB, NIDDK
	Samir Witta	Visiting Fellow	GBB, NIDDK
	Susan Spence	IRTA	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Genetics and Biochemistry Branch

SECTION Mechanisms of Gene Regulation Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 4 PROFESSIONAL: 4 OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

POU transcription factors have been shown to be important in the regulation of cell fate decisions during development. We have focused on understanding the function of one POU domain gene, XlPOU 2 , in early neural development. XlPOU 2 is immediately downstream in the neural inductive signaling pathway for noggin. Our current goal is to identify noggin inducible elements in the XlPOU 2 promoter. XlPOU 2's temporal and spatial pattern of expression suggests that it may be regulatory in the neural inductive process. Misexpressing XlPOU 2 in ventral ectoderm results in a cell fate change from an epidermal to neuronal phenotype, demonstrating XlPOU 2's potential role as a master regulator in neurogenesis. We have examined the role of XlPOU 2 in Spemann's organizer by overexpressing the XlPOU 2 gene product in embryos. The overexpression of XlPOU 2 leads to a suppression of dorsal gene function in gastrulating Xenopus embryos, which results in a "headless" phenotype. We have also characterized a novel POU gene in zebrafish, taichi. The expression pattern of taichi during development and in the adult brain is consistent with its potential role in regulating the establishment and/or maintenance of neural stem cells. Taichi expression is associated with proliferating, non-differentiated cells of the brain. This cell population can be labeled by BrdU, and does not express two markers of neural differentiation, acetylated tubulin and neurofilament protein. We are characterizing these cells further using another marker that is co-expressed with taichi, NRP-1 (an RNA binding protein). To further understand the role of stem cells in mammalian development, we are attempting to clone a mouse homolog of taichi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52021-03 GBB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mapping of triiodothyronine responsive genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Vera Nikodem	Chief, Mech Gene Reg Sect	GBB, NIDDK
Others:	Qianxun Xiao	Visiting Fellow	GBB, NIDDK
	Susan Castillo	IRTA	GBB, NIDDK
	Marcia Phyillaier	Biologist	GBB, NIDDK
	Roland Lippoldt	Special Volunteer	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Genetics and Biochemistry Branch

SECTION Mechanisms of Gene Regulation Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 2.2	PROFESSIONAL: 2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nuclear hormone Nurr1 and Nur77 are members of the steroid/thyroid hormone receptor gene superfamily. They have a conserved amino acid sequence in the zinc-finger DNA binding domains and similar COOH-terminal regions, but have no known ligands. In this study, the regional expression of mRNA encoding these two proteins in rat brain has been determined by in situ hybridization. The Nurr1 mRNA highly expressed in piriform and entorhinal cortex, hippocampus formation, medial habenular and paraventricular thalamus nuclei. Moderate labeling was seen in layer II-V of most cerebral cortex and dorsal lateral geniculate nucleus, substantia nigra (compacta and reticular par) and interpeduncular nucleus. No Nurr1 hybridization signal were seen in the rhombencephalon. In cerebellum, Nurr1 mRNA is present in the internal granular cell layer and Purkinje cell layer. In contrast, Nur77 has a widespread distribution, with the highest level of expression in the cerebral cortex. Moderate hybridization signals were detected in the hippocampus formation and the laterdorsal and posterior, reuniens thalamus nuclei and paraventricular and supraoptic hypothalamus nuclei. In rhombencephalon, higher signal was present in the medial and lateral vestibular, dorsal cochlear and facial nuclei and medial raphe nuclei. Signal was also present in the nucleus of the spinal tract of the trigeminal (V) nerve. In cerebellum, Nur77 mRNA highly expressed in the Purkinje cell layer and lateral deep nucleus of the cerebellum. Our results show that Nurr1 and Nur77 mRNAs have overlapping and different distribution patterns within brain, suggesting that they might regulate different sets of responsive genes. Differences in tissue distribution and developmental expression of these mRNAs suggest that they might regulate different sets of responsive genes. Furthermore, we have identified a truncated isoform of Nurr1, Nurr1a. Comparison of cDNA with genomic sequences revealed that Nurr1a was generated by internal splicing resulting in a frameshift. Nurr1, Nurr1a, Nur77 and 3'-truncated mutants bind in gel-mobility shift assays to the Nurr77 response element. However, in transient transfection assays, a truncation of as little as 15 Nurr1 carboxy-terminal amino acids greatly diminished expression, a result not seen for a similar Nur77 15 amino acid deletion mutant. Thus, an important domain responsible for transactivation of Nurr1 but not Nur77 is located within the last 15 amino acids of the COOH-terminal. Identification of Nurr1a, a transcript which has lost the last 143 amino acids of its COOH-terminal, results in impaired DNA binding and may indicate the use of alternative splicing as a putative mechanism of regulating transcriptional control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52022-03 GBB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Entry into the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harris Bernstein	Sr. Staff Fellow	GBB, NIDDK
Others:	Nancy Ulbrandt	IRTA Fellow	GBB, NIDDK
	John Newitt	IRTA Fellow	GBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand how newly synthesized proteins which must be secreted or inserted into membranes are identified and directed to transport sites in the endoplasmic reticulum (ER) or the cytoplasmic membrane in prokaryotic cells. The project combines biochemical and genetic approaches to investigate the role of the signal recognition particle (SRP), the signal recognition particle receptor (SRP receptor), and their bacterial homologs in this process. Previous studies have shown that the SRP ribonucleoprotein (RNP) recognizes nascent polypeptide chains bearing "signal sequences", which earmark proteins for entry into the ER, and then catalyzes their translocation across the membrane upon interaction with the ER-bound SRP receptor. A principal aim of this project is to elucidate the mechanism by which SRP recognizes signal sequences and then releases them in a regulated manner. By studying this problem we also expect to obtain insight into questions of broad biological significance, such as the regulation of multi-step pathways, the structural features which enable a protein to possess broad substrate specificity, and the functional roles of RNA.

Previous work indicated that signal sequences bind to a site in the C-terminal domain of the 54kd subunit of mammalian SRP (SRP54). Two new lines of evidence, however, suggest that the N-terminal GTPase domain of the protein also plays a significant role in signal sequence recognition. Point mutations introduced into a conserved motif in this domain do not affect GTPase function, but rather reduce signal sequence binding activity. In addition, mutants of the *E. coli* homolog of SRP54 ("Ffh") which were selected on the basis of having moderately reduced signal sequence binding activity contain mutations primarily in the N-terminal domain.

Because of methodological limitations to studying the SRP pathway in mammalian cells, we have begun to study homologs of SRP and the SRP receptor in *E. coli*. The reduced complexity of the SRP pathway as well as the ease of performing genetic experiments in this organism makes it is an attractive model system. The role of SRP homologs in protein export has been controversial in part because substantial evidence suggests that many proteins can be targeted to the secretory pathway in an SRP-independent fashion. We have now obtained strong genetic evidence that SRP targets proteins to the previously identified SecY transport channel. These studies also suggest that inner membrane proteins may be among the substrates which are targeted via the SRP pathway. In other studies we have obtained surprising evidence that the *E. coli* homolog of SRP RNA may function not only in an RNP complex with Ffh, but also either in a complex with another protein or as a free RNA at another stage of the SRP pathway.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-654-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52023-03 GBB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*

Genes ancestral to the thyroid/steriod receptor family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Joseph E. Rall, Senior Scientist, GBB, NIDDK

Others: Zdenek Kostrouch, Visiting Associate, GBB, NIDDK
Marta Kostrouchova, Special Volunteer, GBB, NIDDK

COOPERATING UNITS *(if any)*

University of Acquila, Italy

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to Z01 DK 47033-01 DB.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53100-07 DDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification and characterization of receptors for GI peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	S.A. Mantey	Chemist	DDB, NIDDK
	R. Benya	Clinical Associate	DDB, NIDDK
	T. Kusui	Visiting Associate	DDB, NIDDK

COOPERATING UNITS *(if any)*

Tulane University, New Orleans, LA (D.H. Coy).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Gastroenterology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

I.A.1. Characterization of the gastrin-releasing peptide (GRP) receptor expressed in baculovirus (BV). The GRP receptor is expressed in low amounts in most tissues, and a BV-expression system might allow high expression. We expressed the murine GRP-R (mGRP-R) in SF9 cells using a recombinant BV and characterized it structurally and functionally. High levels of expression were obtained and the receptor was coupled to phospholipase C and to G proteins. The glycosylation was more immature than in native mGRP-R and the expressed BV-mGRP-R had 23% of the mature receptor's glycosylation. The ability of this system to express mGRP-R in increased amounts should be useful for obtaining receptor for antibodies, performing reconstitution studies and GRP-R modulation by agonists.

I.B. Characterization of GI peptide receptors by the development of selective agonists or antagonists.

I.B.1. Characterization of guinea pig chief cell CCK_A receptors using a newly developed selective CCK_A receptor agonist ligand. In many cells CCK_A and CCK_B receptors are both present however it is difficult to study them if CCK_B receptors are present in much larger numbers. Functional studies of the guinea pig chief cell provide evidence for CCK_A and CCK_B receptors, however, the CCK_A receptors have not been able to be identified with existing ligands. We developed a selective, high affinity CCK_A specific ligand. CCK_A receptors were identified on chief cells with this ligand even though present in 1/100 the density of CCK_B receptors. For the first time the relationship between binding and coupling for this cell could be examined with this ligand.

I.B.2. Structural requirements for GRP-R and neuromedin B receptor (NMB-R) antagonists. The GRP-R and NMB-R mediate the actions of these two peptides in mammals. The physiology and pathophysiology of NMB remains unclear because in contrast to the GRP-R no antagonists exist. In this study with Prof. D. Coy, Tulane University, we applied the 5 strategies which yielded GRP-R antagonists to NMB. Neither [desmet^9]NMB amides, esters, alkylamides; φ8-9 NMB analogues or D-amino acid NMB substituted analogues functional as antagonists. Only D-amino acid substituted substance P analogues or somatostatin analogues functional as weak antagonists. These results suggest that these two receptors have markedly different structure-function relationships and novel strategies will be needed to identify NMB-R antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53101 07 DDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular basis of action of gastrointestinal peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
	R.V. Benya	Clinical Associate	DDB, NIDDK
	T. Tsuda	Visiting Fellow	DDB, NIDDK

COOPERATING UNITS *(if any)*

G. Kroog and J. Battey (NIDCD), D.H. Coy (Tulane University).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Section on Gastroenterology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

V. Cellular basis of action of GI peptides.

V.A. Chronic desensitization and down-regulation of the GRP receptor (GRP-R) are mediated by a PKC-dependent mechanism. The GRP-R undergoes chronic homologous desensitization and down-regulation, however, the mechanisms are unclear. To gain insight we prepared 5 mutant receptors: a COOH truncated mutant, a COOH terminal Ser/Thr free mutant, a COOH terminal PKC-consensus sequence (PKC-CS) deleted mutant, and 2 mutants that bound ligand but did not activate PLC. Our results suggested that activation of the distal COOH PKC-CS is essential for chronic desensitization and down-regulation of the GRP-R and provide no evidence for involvement of 2nd messenger independent processes. In contrast, internalization is equally regulated by both 2nd messenger-dependent and independent processes.

V.B. The GRP receptor is phosphorylated by a non-PKC kinase after agonist exposure. Many protein-coupled receptors are phosphorylated post agonist exposure which modulates receptor activity. We investigated agonist-induced phosphorylation of the GRP-R using immunoblotting with a GRP-R antibody. Agonist-induced phosphorylation was rapid and we provide evidence that it is not caused by PKC-activation.

V.C. Peptide structured requirements for high affinity interaction with the two mammalian bombesin receptors. There is minimal information on the peptide structural features determining specificity for the GRP-R or NMB-R. In the present study we used transfected cells possessing either receptor and examined the ability of natural occurring Bn-like peptides, NMB and Bn COOH terminal fragments and NMB analogues made more GRP-like by single amino acid substitutions (positions 3,6,9) as well as various conformationally restricted analogues to interact with each receptor subtype. Our results suggest that the active conformation of NMB differs markedly from the folded-β-sheet model proposed for GRP, the structure-function differ markedly for the 2 receptors and suggest the NH_2 terminus of GRP and NMB is important for receptor selectivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53200-04 DDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and management of islet cell tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	D.B. Strader	Clinical Associate	DDB, NIDDK
	M. Orbuch	Clinical Associate	DDB, NIDDK
	B. Termanini	Clinical Associate	DDB, NIDDK

COOPERATING UNITS *(if any)*

National Cancer Institute, Surgery Branch, NIH (D. Fraker); Radiology Department, NIH (J.L. Doppman); Biostatistics and Data Management, NCI, (D. Venzon).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Gastroenterology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	3.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

III.A. Natural history and determinants of survival in patients with ZES. Little is known about the natural history or determinants of long term survival in patients with malignant pancreatic endocrine tumor syndromes. In collaboration with Dr. David Venzon, NCI, these parameters were analyzed in 185 patients with ZES. Tumor size, location, presence or absence of MEN-1 were all found to be factors in determining metastatic rate to the liver which was the primary determinant of survival. Two clinical forms of ZES a benign and malignant form were identified. This study for the first time identifies clinical parameters that can be used to determine the natural history and used to develop specific treatments.

III.B. Possible benefit of surgical resection in gastrinoma patients. Controversy exists whether patients with ZES should undergo surgical exploration. In collaboration with the Surgical Metabolism Section, NCI, the longterm courses of patients with ZES who did and did not undergo surgical exploration for possible cure were analyzed. A significantly lower ($p<0.003$) rate of development of hepatic metastases in resected patients support the need for surgery in gastrinoma patients and other PET's.

III.C. Prospective study of surgical resection of gastrinomas in MEN-1. The surgical treatment of patients with ZES and MEN-1 is controversial. A recent study proposes the gastrinoma is frequently duodenal in location and is curable. With Dr. D. Fraker, NCI, 10 such patients were studied. Not all gastrinomas were duodenal in location (70%). Furthermore, no patients were curable either because of multiple tumors or lymph node metastases. This study shows that ZES cannot be cured in patients with MEN-1 with simple gastrinoma enucleation or resection.

III.D. Intrahepatic arterial stimulation (IAS) with secretin in localizing metastatic gastrinomas. Identification of liver metastases is essential in the management of patients with pancreatic endocrine tumors (PET's), however, it may be difficult. We have recently developed methods for finding primary tumors measuring hormone gradients after provocative agents. With Dr. John Doppman, Diagnostic Radiology Department, we prospectively studied this method for identifying hepatic metastases in patients with ZES. Using established criteria, IAS was helpful in 22% of the 74 patients studied. This methodology with other provocative agents may be useful in PET's in resolving hepatic involvement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53201-06 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors on gastric smooth muscle cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	T. Pradhan	Chemist	DDB, NIDDK
	Z-F. Gu	Visiting Fellow	DDB, NIDDK

COOPERATING UNITS *(if any)*

Peptide Research Lab, Tulane University, (Drs. Rossowski and Coy).

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Merged with project no. Z01 DK 53203-01 DDB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53202-04 DDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular characterization of receptors for GI peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	S.A. Wank	Senior Investigator	DDB, NIDDK
	J. Pisegna	Clinical Associate	DDB, NIDDK
	R. Benya	Clinical Associate	DDB, NIDDK
	M. Pohl	Visiting Fellow	DDB, NIDDK
	S. Poirot	Visiting Fellow	DDB, NIDDK

COOPERATING UNITS *(if any)*

K. Huppi, LG, MGS, NCI; J.F. Battey, J.D. Gardner, St. Louis University, Department of Medicine.

LAB/BRANCH

Digestive Diseases Branch

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.7	5.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

II. Molecular characterization of GRP receptors.

II.A. Multiple affinity state of different cholecystokinin (CCK) receptors. CCK receptors are reported to exist in different affinity studies. To characterize these states, agonist and antagonist binding was performed on cells transfected with rat CCK-A-R, rat CCK-B-R, canine CCK-B-R and a [$L^{355}V$]canine CCK-B-R mutant. Each receptor could exist in 3 affinity states and the ability to exist in multiple states was an intrinsic property of the CCK receptor.

II.B. Expression and characterization of cloned human bombesin (Bn) receptors. Little is known about the pharmacology or cellular basis of action of human Bn receptors. The human GRP-R (hGRP-R) and hNMB-R were stably transfected into BALB/3T3 fibroblasts and characterized by pharmacological studies--cross-linking and intracellular activation pathways explored. These transfected cells exhibit similar pharmacology, glycosylation and changes in biologic activity with agonists to natively expressed receptors and should be useful to explore further the cellular basis of action of these peptides in humans.

II.C. Chromosomal localization of CCK-A-R and CCK-B-R in humans and mouse. The CCK-A-R maps to a synteric region of human chromosome 4 and mouse chromosome 5. The CCK-B-R resides on a synteric region of human chromosome 11 and distal mouse chromosome 7. The CCK-receptors localize with 2 dopamine receptors DRD5 and DRD4 raising the possibility of coinvolvement in CNS or neuropsychiatric illness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53203-01 DDB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Action of GI hormones on gastric smooth muscle cells/gastric chief cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Jensen	Branch Chief	DDB, NIDDK
Others:	T. Pradhan	Chemist	DDB, NIDDK
	Vito Corleto	Visiting Fellow	DDB, NIDDK

COOPERATING UNITS *(if any)*

Peptide Research Lab, Tulane University, (Drs. Rossowski and Coy).

LAB/BRANCH

Digestive Diseases Branch

SECTION

Section on Gastroenterology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

IV.A. Actions of GI hormones on gastric chief cells.

IV.A.1. Action of bombesin on gastric chief cells. Bombesin-related peptides *in vivo* stimulate pepsinogen release however it is unclear whether it is due to a direct action on chief cells or an indirect action. To attempt to address this question, we studied the action of bombesin-related peptides on isolated guinea pig chief cells. Bombesin-related peptides (Bn, GRP, NMB) did not stimulate pepsinogen release, alter $[Ca^{2+}]$, cAMP or inositol phosphate levels alone or effect stimulation by other agents. Very low levels of binding were seen suggesting binding to contaminating cells. These results demonstrate bombesin related peptides do not alter pepsinogen release by direct interaction with chief cells.

IV.B. Actions of GI hormones on gastric smooth muscle cells.

IV.B.1. Somatostatin (SS) receptor subtypes on gastric smooth muscle cells. Gastric smooth muscle cells are shown to possess high affinity SS receptors. Smooth muscle cells rapidly degraded SS-14, however with appropriate protein inhibitors present and the use of a peptidase resistant ligand the pharmacology and stoichiometry of the SS receptors were explored. The receptor exists in 2 affinity states which are regulated by a G-protein. In collaboration with Dr. D.H. Coy (Tulane University) using SS receptor subtype specific analogues, the action of SS on these cells was shown to be mediated by the SSTR-3 subtype.

IV.2. Mechanism of galanin-induced relaxation. Recently, GI smooth muscle cell relaxants have been shown in some cases to both activate adenylate cyclase and NO synthetase. In isolated guinea pig gastric smooth muscle cells both VIP and galanin caused equipotent relaxation. Using NO synthetase and PKA inhibitors our results demonstrate galanin's relaxant effect is mediated only by cAMP whereas VIP both activates adenylate cyclase and nitric oxide synthetase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53501-19 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Studies Relating to Pathogenesis of Hepatic Encephalopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. : Nora Bergasa Senior Staff Fellow LDS, DDB, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Neuroscience, NIDDK (P. Skolnick and A.S. Basile), Mental Health, DIR, NIMH (S.M. Paul), National Institute of Drug Abuse, Baltimore (R.B. Rothman)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53511-14 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trials of Therapies for Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. :	J.H. Hoofnagle	Director	DDDN, NIDDK
Others:	H. Conjeevaram	Staff Fellow	LDS, NIDDK
	D. Herion	Staff Fellow	LDS, NIDDK
	R. Sallie	Visiting Associate	LDS, NIDDK
	D. Lau	Visiting Associate	LDS, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Neuroscience, NIDDK (P. Skolnick and A.S. Basile), Mental Health, DIR, NIMH (S.M. Paul), National Institute of Drug Abuse, Baltimore (R.B. Rothman)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary biliary cirrhosis (PBC) is a progressive liver disease believed to be of autoimmune nature. Its etiology is unknown. It is characterized by progressive intrahepatic cholestasis as a result of onging non-suppurative destructice cholangitis that affecting small intrahepatic bile ducts. It affects approximate 1 in 2000 woen above the age of 50 and it os one of the 3 or 4 major reasons for liver transplantation in the U.S. Several trials have been conducted to study the effect of immunosuppressants in PBC including methotrexate (MTX). A randomized trail comparing th effects of 7.5 vs 15 mg per week of oral MTX was started in 1991 and completed in early 1995. 28 patients were randomized to receive one of the two doses and stratified according to the presence or absence of symptoms. Therapy led to decreases in serum alkaline phosphatase and aminotransferase levels and fall to IgM levels to normal. Symptoms improved in 43% of patients and liver histology in 46%. The higher dose was associated with greater improvements in liver histology but the lower dose was effective in a similar percentage of patients. These results indicate that prolonged therapy with adjusted doses of MTX may be effective in ameliorating symptoms and liver pathology in PBC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 53516-04 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Studies of the Opiate System in Cholestatic Liver Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Nora Bergasa, Ph.D. Senior Staff Fellow LDS, NIDDK

COOPERATING UNITS *(if any)*

NHLBI (Dr. S. Sabol, Dr. W. Scott Young), DIR, NIAID (Dr. David Alling), Biomedical Engineering (Thomas Talbot and Eli Walker)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐(b) Human tissues ☐(c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 54001-04 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Natural History and Treatment of Chronic Hepatitis B

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: J.H. Hoofnagle, Director, DDDN, NIDDK
H. Conjeevaram, Staff Fellow, LDS, NIDDK
P. Hayashi, Staff Fellow, LDS, NIDDK
R. Sallie, Visiting Associate, LDS, NIDDK
D. Lau, Visiting Associate, LDS, NIDDK
A.O. Shakil, Visiting Associate, LDS, NIDDK
M. Beames, Lab Technician, LDS, NIDDK
J. Vergalla, Lab Technician, LDS, NIDDK

COOPERATING UNITS *(if any)*

E. Tabor, DCE, NCI, R. McKenzie and S. Straus, LCI, NIAID. J. Gerin, Georgetown Univ. Feinstone, CBER, FDA; F. Chisall, Scripps Institute, San Diego, CA.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.8	5.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection with hepatitis B virus is a major cause of liver disease worldwide and accounts for 5-10% of chronic liver disease and cirrhosis in the United States. Safe and effective vaccines are now available for prevention of hepatitis B. However, therapies for the disease once it has ocurred are unsatisfactory. Alpha interferon was the first antiviral agent shown to be effective in this disease and was licensed for this indication in the United States in 1993. The bases for licensure were, in part, studies conducted in the Liver Diseases Section of the NIH. Unfortunately, alpha interferon is effective in only one-third of patients with typical chronic hepatitis B and its role in atypical forms of this disease remain unclear. Current activities in the Liver Diseases Section are focused on understanding the immunopathogenesis of chronic HBV infection and developing better therapies to prevent in longterm consequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 54002-04 DDB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*
Studies of the Natural History and Treatment of Chronic Type C Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: J.H. Hoofnagle, Director, DDDN, NIDDK
H. Conjeevaram, Staff Fellow, LDS, NIDDK
P. Hayashi, Staff Fellow, LDS, NIDDK
R. Sallie, Visiting Associate, LDS, NIDDK
A.O. Shakil, Visiting Associate, LDS, NIDDK
M. Beames, Lab Technician, LDS, NIDDK
J. Vergalla, Lab Technician, LDS, NIDDK
M. Battegay, Guest Researcher, LDS, NIDDK

COOPERATING UNITS *(if any)*
H. J. Alter, Dept. Transfusion Medicine, CC, NIH. S.M. Feinstone, CBER, FDA. K. Krawczynski, Hepatitis Branch, CDC

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	4.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with well-documented chronic hepatitis C are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients are available to evaluate experimental therapies. Previous studies have shown that alpha interferon therapy has a short-term beneficial effect in approximately 50% of patients. This response is sustained in approximately 20% after stopping interferon. A randomized, placebo-controlled trial of ribavirin showed that a 1 year course of this nucleoside analogue is associated with significant improvement in serum aminotransferase activities and in hepatic lobular necrosis despite unchanged serum levels of HCV RNA. Continous ribavirin therapy for up to 2 years demonstrates the practicality of long term suppresssive therapy in this disease. A controlled trial of the combination of ribavirin and interferon has been designed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 55000-23 MCEB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis, Glycosylation, and Action of Thyrotropin: Clinical Trials of Recombinant TSH

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. D. Weintraub	Chief	MCEB, NIDDK
Others:	M.W. Szkudlinski	Staff Fellow	MCEB, NIDDK
	J.E. Tropea	Postdoctoral IRTA Fellow	MCEB, NIDDK
	J. East-Palmer	Biologist	MCEB, NIDDK
	M. Grossmann	Visiting Fellow	MCEB, NIDDK

COOPERATING UNITS (if any)

None

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.5 PROFESSIONAL: 3.5 OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recombinant thyrotropin which is used to stimulate radioiodine uptake in patients with thyroid cancer has been produced by novel methods of transfection of alpha and hTSH beta minigenes into Chinese hamster ovary cells. Utilizing products produced in a large scale bioreactor as well as a hollow fiber bioreactor, we have been able to purify several hundred milligrams of this product. Terminal sialylation of recombinant TSH determines in vitro and in vivo bioactivity as well as clearance rate. The carbohydrate structure of recombinant TSH has been characterized in detail and differs from pituitary TSH. The role of sugar structures in bioactivity has also been clarified by enzymatic deglycosylation.

Site-directed mutagenesis as well as production of novel recombinant hybrids has been used to develop new TSH analogs with prolonged half life. We have also developed competitive antagonists of TSH which will be useful in treatment of patients with hyperthyroidism and thyroid cancer. Using homology modeling of the structure of recombinant TSH, we have been able to design superagonists based on rational principles. Such superagonists are active in a variety of in vitro and in vivo assays and and include other glycoprotein hormones such as gonadotropins.

We have completed phase 1-3 clinical trials of recombinant TSH in collaboration with 12 other medical centers. After one additional confirmatory trial, this drug should be approved for the diagnosis of thyroid cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55002-15 MCEB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Pituitary Glycoprotein Hormones and Hypothalamic Releasing Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCEB, NIDDK
Others:	M. K. Kim	Senior Staff Fellow	MCEB, NIDDK
	L. A. Lesoon-Wood	IRTA Postdoctoral Fellow	MCEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.5

PROFESSIONAL: 2.5

OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human TSH beta gene is expressed only in the thyrotrophs of the anterior pituitary where it is regulated by hypothalamic hormones, cyclic AMP as well as thyroid hormone. Recent studies have indicated that TRH and cyclic AMP mediate their transcriptional action in large part through changes in phosphorylation of the specific pituitary factor pit-1 which binds to a region upstream of the start of transcription. In contrast, thyroid hormone mediates its inhibition through binding to a regulatory element downstream of the start of transcription in the first untranslated exon.

We have also characterized in detail a TGGGTCA motif at -1/+6 of the hTSH-beta gene that mediates, in conjunction with the upstream pit-1 site, protein kinase C and A as well as TRH stimulation of hTSH-beta gene expression. We have also shown that c-fos and c-jun which interact with this downstream site modulate T3 inhibition of hTSH-beta gene expression.

We have recently recognized other important regulatory sequences for hTSH-beta gene expression. One such area is a pituitary-specific TATAAA sequence at -25 which is vital for both basal and regulated expression. Other regions include unique silencer regions at two locations upstream in the promoter. These regions include Oct-1 binding sites and appear to act as nuclear matrix attachment regions.

Currently, studies are in progress to characterize fully the silencer region and determine how it interacts with both stimulatory and inhibitory regulatory regions. Ultimately, interaction of these regions with the basal transcription machinery will be elucidated.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01DK55006-22MCEB

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin-like Growth Factor Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. M. Rechler	Chief, Growth & Development Section	MCEB, NIDDK
	Y. Boisclair	Visiting Associate	MCEB, NIDDK
Others:	F. Cohen	Med. Staff Fellow	MCEB, NIDDK
	S. Hsieh	Pre-IRTA	MCEB, NIDDK
	C-Y. Lee	Visiting Fellow	MCEB, NIDDK
	G.T. Ooi	Visiting Associate	MCEB, NIDDK
	D. S. Suh	Visiting Fellow	MCEB, NIDDK
	L. Tseng	Chemist	MCEB, NIDDK
	H. Wu	Visiting Fellow	MCEB, NIDDK
	Y. Yang	Sr. Staff Fellow	MCEB, NIDDK
	Y. Zhou	Visiting Associate	MCEB, NIDDK

COOPERATING UNITS *(if any)*
Bone Research Branch, NIDR, NIH (M. Yanagishita); Cornell University, Ithaca, NY (Y. Boisclair); Austin Hospital, Heidelberg, Victoria, Australia (L.A. Bach)

LAB/BRANCH Molecular & Cellular Endocrinology Branch

SECTION Growth & Development Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892-1758

TOTAL MAN-YEARS: 9.00	PROFESSIONAL: 8.00	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to investigate how insulin-like growth factor binding proteins (IGFBPs) modulate the biological actions of IGF-I and IGF-II. The IGFs occur in plasma, other extracellular fluids, and tissues complexed to one or more members of a family of six IGFBPs. The IGFBPs determine the bioavailability of the IGFs and may inhibit or potentiate IGF action. As they have distinct properties and are differently regulated, the IGFBPs provide a flexible and versatile regulatory system. In the past year, we have studied the: (i) regulation of IGFBP-1 promoter activity in rat hepatoma cells by glucocorticoids and insulin; (ii) structure and regulation of the gene for the acid-labile subunit (ALS) of the 150 kDa IGFBP complex in plasma; (iii) formation of the 150 kDa ALS:IGFBP-3 complex in plasma; and (iv) binding of IGFBP-3 to human fibroblasts and rat glioma cells. Noteworthy results include: (i) Rat IGFBP-1 promoter. A glucocorticoid response element (GRE) and an insulin response element (IRE) have been identified in the proximal region of the rat IGFBP-1 promoter that are essential for the stimulation of promoter activity by dexamethasone, and the rapid inhibition of promoter activity by insulin, respectively. Coordinated action of liver-enriched and hormonally regulated transcription factors binding to several sites is required for both basal and dexamethasone-stimulated promoter activity. (ii) Structure and regulation of the ALS gene. ALS mRNA is synthesized predominantly in liver, and regulated by growth hormone (GH). The mouse ALS gene contains a single intron. Constructs suitable for introducing an inactive ALS gene into the mouse genome by homologous recombination have been prepared. GH regulates ALS promoter activity in a rat hepatoma cell line but not in GH-responsive mouse preadipocytes. (iii) Formation of plasma 150 kDa complexes, and release of IGF-I from the complexes by proteolysis of IGFBP-3. In addition to ternary complexes of ALS, IGFBP-3 and IGF-I, adult rat serum also contains 150 kDa binary complexes of ALS and proteolytically-nicked IGFBP-3 without bound IGF. We have shown that these complexes can form by direct association of ALS and IGFBP-3, and by proteolysis of IGFBP-3 within 150 kDa ternary complexes with subsequent release of IGF-I. Proteolysis of IGFBP-3 within 150 kDa complexes by cation-dependent serine proteasess provides a mechanism by which IGFs can be mobilized from their reservoir in the circulation. (iv) Binding of IGFBP-3 to human fibroblasts and rat glioma cells. Although IGFBP-3 binding to human fibroblasts and rat glioma cells is inhibited by heparin, IGFBP-3 does not bind to structurally similar heparan sulfate proteoglycans on the surface of these cell types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK55015-07 MCEB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutations of the Thyroid Hormone Receptor Gene in Patients with Thyroid Hormone Resistance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.D. Weintraub, M.D.	Chief	MCEB, NIDDK
Others:	R. Wong	Visiting Associate	MCEB, NIDDK
	M. B. Grace	Predoctoral IRTA Fellow	MCEB, NIDDK
	F. Davis	Visiting Associate	MCEB, NIDDK
	D.-W. Gong	Visiting Fellow	MCEB, NIDDK

COOPERATING UNITS (if any)

Dr. S.-y. Cheng, Senior Investigator, Laboratory of Molecular Biology, National Cancer Institute

LAB/BRANCH Molecular and Cellular Endocrinology Branch

SECTION Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Resistance to thyroid hormone (RTH) is an inherited disease linked to mutations in the beta T3 receptor gene and characterized clinically by the resistance of peripheral and pituitary tissues to the action of thyroid hormone. Of the over 50 different mutations identified in the ligand binding domain of the beta receptor, several were shown to inhibit normal receptor function by a dominant negative mechanism which is likely to mediate the phenotype of this disease. Recent studies have indicated that this dominant negative effect is most likely mediated by competiton of mutant and normal receptor for binding to T3 response elements within various thyroid hormone responsive genes.

We have recently shown that natural thyroid hormone response elements (TRE's) show variation in the dominant negative effect compared to previously employed artificial TRE's. These differences appear to explain the diverse clinical features of RTH. We have recently developed a transgenic model of RTH which should be useful in understanding the function of mutant receptors in vivo and in developing new therapies for the disease. This transgenic mouse model shows selective loss of white adipose tissue as well as hyperactivity. Currently, we are investigating the molecular basis for such phenotypic features and are exploring new aspects of pharmacologic therapy for hyperactivity. Finally, we have developed novel methods to screen for mutations in the receptor to identify mutations rapidly using automated DNA sequencing. All of these advances should be applicable to other dominant genetic diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57501-19 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Function of DNA Virus Genomes in Animal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Roland A. Owens	Senior Staff Fellow	LMCB:NIDDK
Other:	Sirkka R.M. Kyöstiö	IRTA Fellow	LMCB:NIDDK
	Scotty L. Walker	IRTA Fellow	LMCB:NIDDK
	Ramani S. Wonderling	IRTA Fellow	LMCB:NIDDK

COOPERATING UNITS (if any)

R. Kotin, J. Chiorini, B. Safer, S. Wiener (MH/NHLBI/NIH); B. Carter (Targeted Genetics Inc.); M. Weitzman (Institute for Human Gene Therapy, Wistar Institute, U. Penn.); J. Trempe (Medical College of Ohio)

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Rep proteins of Adeno-associated virus type 2 (AAV) have potential as anti-cancer and anti-HIV therapeutics. The AAV rep gene open reading frame encodes four overlapping Rep proteins. Rep68 and Rep78 bind to the AAV genome's inverted terminal repeats, have enzymatic activities which are necessary for AAV DNA replication, regulate AAV gene expression, inhibit HIV-1 replication, are required for preferential integration of the AAV genome into a region of human chromosome 19, and inhibit cell division. Wild-type and mutant Rep proteins were expressed from the HIV-1 long terminal repeat promoter in human 293 cell transient transfections or as maltose binding protein fusions in Escherichia coli. The ability of Rep proteins to down-regulate AAV promoters is optimal when the promoter contains a Rep binding site and the Rep proteins contain a wild-type ATP binding site. Although Rep68 binding to DNA is enhanced by the presence of ATP, wild-type Rep68 can still down-regulate, to a lesser degree, promoters which show no stable Rep binding in the presence or absence of ATP. Based on the consensus binding sequence and electrophoretic mobility shift assays, binding sites for Rep proteins have been identified within several human genes, some of which are known to be involved in the regulation of cell proliferation. A study has been initiated to determine the impact of Rep proteins on the expression of these genes. We have observed a correlation between the ATP-dependent helicase activity of wild-type and mutant Rep proteins and their ability to efficiently down-regulate the p5 and p19 promoters of AAV. Further studies have revealed that mutant Rep proteins with a defect in the ATP binding site are dominant-negative for p5 and p19 down-regulation, DNA helicase activity, and RNA/DNA helicase activity. These observations are consistent with a model in which Rep proteins function as multimers and can down-regulate genes via their helicase activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57502-22 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Regulation of Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Takami Oka	Senior Investigator	LMCB:NIDDK
Other:	Robert Moore	IRTA Fellow	LMCB:NIDDK
	Tetsuo Murakami	Special Volunteer	LMCB:NIDDK
	John W. Perry	Biologist (Technician)	LMCB:NIDDK
	Hiroshi Saito	Visiting Fellow	LMCB:NIDDK
	Li-Xin Zhou	Visiting Fellow	LMCB:NIDDK
	Toshio Maeda	Special Volunteer	LMCB:NIDDK

COOPERATING UNITS *(if any)*

Dr. Inder K. Vijay, University of Maryland, Molecular Biology Center, College Park, Md. Dr. Koichi Enomoto, Shimane Medical University, Japan, Dr. Sergio Lavandero, Department of Molecular Biology, University of Chile, Chile, Dr. Seiji Nishikawa, Department of Periodontology, University of Tokushima, Japan

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Cell Growth and Differentiation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of our current research is to elucidate the molecular mechanism(s) of tissue- and stage-specific, multi-hormone regulated gene expression using the β-casein gene as a tool. β-Casein is the major milk protein synthesized by the mammary gland during lactation. The expression of the mouse β-casein gene is regulated both positively and negatively by the interaction of various peptide and steroid hormones during mammary gland development. Insulin (I), glucocorticoid (H) and prolactin (PRL) are essential for the induction of casein gene transcription and progesterone and EGF inhibit the induction process. PRL exerts its biological effect via a plasma membrane receptor. PRL receptor (PRL-R) exists in at least two forms: a short form (PRL-RS) and a long form (PRL-RL). In the mouse, cDNAs for PRL-RL and three variants of PRL-RS that diverge in the cytoplasmic domains have been isolated and cloned from the mammary gland and the liver, respectively. The two forms differ only in their cytoplasmic domain. Transfection experiments using cDNAs for PRL-R indicated that PRL-RL but not PRL-RS can transduce a PRL signal to stimulate the transcription of casein gene in Chinese hamster ovary cells. To study the mechanism of PRL-R-mediated casein gene expression in the mammary gland, we investigated the regulation of PRL-RL mRNA and its relationship to β-casein gene expression during development of the mammary gland in vivo and in vitro. We found that mouse mammary epithelium expresses only PRL-RL mRNA and that its level is increased during lactation in vivo and by the synergistic actions of I, H and PRL in vitro. Furthermore, we showed that both progesterone and EGF, negative regulators of casein gene expression, inhibit PRL-RL gene expression both in vivo and in vitro. These results indicate that regulation of RRL-RL mRNA is an important step in hormonal induction of milk protein gene expression in the mammary gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57503-22 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lysosomal Transport and Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Frank Tietze

COOPERATING UNITS *(if any)*

William A. Gahl, Section on Human Biochemical Genetics, NICHD

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated on December 30, 1994.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 57800-04 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Initial Intracellular Events of Steroid Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.S. Simons, Jr.	Chief, Steroid Hormones Section	LMCB:NIDDK
Other:	Suzanne F. Bayly	Visiting Fellow	LMCB:NIDDK
	Clayton D. Collier	IRTA Fellow	LMCB:NIDDK
	David A. Jackson	IRTA Fellow	LMCB:NIDDK
	Kevin J. Modarress	IRTA Fellow	LMCB:NIDDK
	Marc E. Poirot	Special Volunteer	LMCB:NIDDK
	Seung H. Shin	Student IRTA	LMCB:NIDDK
	Daniele Szapary	Visiting Associate	LMCB:NIDDK
	Min Xu	Visiting Fellow	LMCB:NIDDK

COOPERATING UNITS *(if any)*

D.P. Edwards (Univ. of Colorado, Denver), W.B. Pratt (Univ. of Michigan Medical School, Ann Arbor), K.R. Yamamato (UCSF, San Francisco), S. Rusconi (Univ. of Fribourg, Switzerland)

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Steroid Hormones Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.5	8.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to define the initial, intracellular events of glucocorticoid hormone action and steroid hormone action in general. Such studies are of particular current relevance since steroid hormone receptors are arguably the best understood regulators of eukaryotic gene transcription. The first step of steroid binding to the intracellular receptor is followed by activation of the receptor-steroid complex to a DNA/nuclear-binding species that then associates with those nuclear acceptor sites involved in the regulation of transcription of selected genes in specific cells. We have concentrated on the nuclear localization of glucocorticoid receptors and how these nuclear receptors influence gene transcription. Glucocorticoid receptors are predominantly cytoplasmic in the absence of steroid and several lines of evidence suggest that microtubules may be intimately involved in the relocalization of steroid-bound glucocorticoid receptors from the cytoplasm to the nucleus. However, this hypothesis is now discredited by our finding that microtubule disruption with micromolar concentrations of colchicine had no effect on nuclear translocation as assayed by the induction of a transiently transfected reporter gene by endogenous receptors. The mechanism of how the nuclear receptor-steroid complexes regulate gene transcription is becoming increasingly complicated with the discovery of new DNA elements and trans-acting factors. We had previously reported that glucocorticoid induction of the tyrosine aminotransferase (TAT) gene involves a novel cis-acting element, called a glucocorticoid modulatory element (GME). We now find that two apparently novel proteins of 88 and 67 kDa bind to this GME in a manner that correlates with the expression of GME activity. Another new, cis-acting element of the TAT gene has now been defined that can block the effects of the GME. These new elements and binding proteins offer additional mechanisms for modifying glucocorticoid regulated gene expression. Collectively, our findings contribute to our long term goal of defining the action of steroid hormones at a molecular level and of understanding their role in human physiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57801-03 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Nancy G. Nossal	Chief, LMCB	LMCB:NIDDK
Other:	Lisa Hobbs	IRTA Fellow	LMCB:NIDDK
	David Wheeler	IRTA Fellow	LMCB:NIDDK
	Medha Bhagwat	IRTA Fellow	LMCB:NIDDK

COOPERATING UNITS *(if any)*

Drs. Craig Hyde and Tim Meuser, LSBR/NIAMS, Dr. Stephen Benkovic, Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing our study of the *E. Coli* bacteriophage T4 model system for duplex DNA replication in which efficient DNA replication *in vitro* is achieved with purified proteins encoded by T4 phage: T4 DNA polymerase (gene 43), gene 32 DNA helix-destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, the genes 41, 61, and 59 primase-helicase, RNase H, and DNA ligase.

Structure of the T4 DNA replication proteins. We are collaborating with Tim Meuser and Craig Hyde, NIAMS, to determine the structure of each of the T4 DNA replication proteins using single crystal X-ray diffraction. The structure of T4 RNaseH, a 5' to 3' RNA:DNA and DNA:DNA nuclease, has been solved by heavy atom MIR to 2.8Å for an orthorhombic crystal form. A monoclinic form of higher resolution was then solved by molecular replacement using the refined model. The difference electron density map of the orthorhombic crystal form with $BaCl_2$ replacing $MgCl_2$ clearly shows a single barium binding site in the putative active site of the enzyme, surrounded by the amino acids that are most highly conserved in the prokaryotic and eukaryotic nucleases related to T4 RNaseH. We are constructing single site mutations to test our model and produce an inactive enzyme to crystalize with its duplex substrates. Two orthorhombic crystal forms of the gene 59 helicase assembly protein diffract to high resolution. *Function and structure of the T4 primase-helicase complex.* We are studying the mechanism by which the gene 59 protein stimulates DNA unwinding by the 41 helicase, and primer synthesis dependent on both the 41 and 61 proteins, using affinity chromatography, gel filtration, and chemical cross-linking. ATP increases the cross-linking of both the 41 and 59 proteins to photoactivatable DNA substrates, and cannot be replaced by ATPγS; 59 and 41 proteins each cross-link to the DNA to a much greater extent in the presence of the other protein. *Structure and function of the 737-772 region of T4 DNA polymerase.* Our structural analysis suggests that the 737-772 region, previously been shown to have effects on polymerase fidelity, may represent a pair of helices bent at G753, and interacting intramolecularly with one another via hydrophobic interactions, or alternatively, could be a dimerization domain between two polymerases. Site-direct mutagenesis is being used to test predictions of these models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57802-08 LMCB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacteriophage T4 Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Deborah M. Hinton	Research Chemist	LMCB:NIDDK
Other:	Mridula Sharma	Visiting Associate	LMCB:NIDDK
	Philip Marshall	Visiting Fellow	LMCB:NIDDK
	Jeffrey Gerber	Pre-IRTA Fellow	LMCB:NIDDK
	Sujal Shah	Pre-IRTA Fellow	LMCB:NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study a simple bacteriophage system, the progression of promoter usage after infection by bacteriophage T4, as a model for investigating mechanisms of transcriptional regulation. During infection, different classes of T4 promoters (early, middle, late) are recognized as infection proceeds. Middle transcription is performed by the host RNA polymerase, which is modified by ADP-ribosylation and by the binding of the T4 AsiA protein to sigma70, the specificity subunit of RNA polymerase. Middle transcription by modified polymerase also requires the T4 transcriptional activator, MotA, which binds to the -30 region of the promoter. Curiously, unmodified polymerase alone can transcribe from middle promoters, but it is not activated by MotA. By investigating transcription complexes made by unmodified polymerase and T4-modified polymerase, we have found that unmodified polymerase requires only the far downstream sequence elements of a middle promoter (the -10 region). The phage modifications to the polymerase alter this recognition, resulting in a polymerase that now requires the binding of MotA to the -30 region in order to unwind the DNA and initiate transcription. We have cloned the asiA gene, purified the AsiA protein, and demonstrated that the presence of AsiA is the only modification required for MotA activation. The protein-DNA complex made in the presence of MotA, AsiA and polymerase is more stable to electrophoresis than that made in the absence of any one of these components. These results suggest that the stability of the modified polymerase-promoter complex is mediated through AsiA. We have investigated the importance of different regions of the T4 middle promoter PuvsX, which has MotA boxes at -30, -35, -51, -70, and -87. We constructed mutant PuvsX promoters and assayed the mutants for transcription and binding. Our results indicate that MotA binds independently to the boxes at -30 and -51, and suggests that the box at -35 is not important for MotA binding. Promoters with changes to the -51 or -35 MotA boxes are still activated by MotA, but the promoter lacking a MotA box at -30 is inactive, indicating that this binding site is critical for transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK58000-50 LAC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analytical Service and Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edwin D. Becker	Acting Chief, LAC	LAC/NIDDK
Others:	Herman J. C. Yeh	Research Chemist	LAC/NIDDK
	Lewis K. Pannell	Visiting Scientist	LAC/NIDDK
	Noel Whittaker	Chemist	LAC/NIDDK
	Wesley White	Biologist	LAC/NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Bioorganic Chemistry, NIDDK; Laboratory of Medicinal Chemistry, NIDDK; Laboratory of Biophysical Chemistry, NHLBI; Laboratory of Experimental Pathology, NCI

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Instrumentation Section

INSTITUTE AND LOCATION

NIH/NIDDK, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to NIDDK scientists, other NIH laboratories and, to a limited extent, personnel of other government agencies. Instrumental analyses include nuclear magnetic resonance of liquids and solutions; infrared spectroscopy of solids, liquids, and samples introduced by gas chromatograghy; mass spectrometry (with samples introduced by solids probe, gas and liquid chromatography and capillary zone electrophoresis and ionization by electron impact, chemical ionization, electrospray, thermospray, thermabeam, and fast atom bombardment)

Requests for analyses are obtained from many laboratories and Institutes at NIH, as well as some outside organizations. The laboratory in this way acts as an NIH resource and it is supported, in part, by its collaborators in terms of staffing and equipment where appropriate. Some major instruments are jointly funded and shared with other Institutes to avoid costly duplication of facilities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER Z01 DK58012-03 LAC

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Application of NMR Spectroscopy in Chemical and Biochemical Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Herman Yeh	Research Chemist	LAC, NIDDK
Xiaohong Liu	Visiting Fellow	LAC, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Bioorganic Chemistry,NIDDK; Department of Chemistry, Purdue Univ.

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Instrumentation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various NMR techniques have been provided to laboratories and collaborating groups in solving the solution structures of biomolecules. Using these techniques in conjunction with computer modelling we have investigated solution structures of DNA duplexes modified at the adenosine residues with (±)diol epoxide metabolites of benzo[a]pryene (in collaboration with Dr. D.M. Jerina's group). These studies reveal that for the dA adducted DNA the BaP is intercalated in the interior of DNA duplex. This is in contrast to the dG adducted DNA which shows that BaP is exposed at the exterior of DNA duplex. Current results also indicate that chirality at the site of attachment influences the orientation of the hydrocarbon as well as the glycosidic torsion angle at the modified deoxyadenosine.

Collaborative interest has continued in the structural determination of alkaloids isolated from natural products (in collaboration with Dr. J. Daly's group). A personal computer has been added and networked to the VXR 500 data system which allow annotation of NMR spectra.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER Z01 DK58013-03 LAC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mass Spectrometry of Drugs, Natural Products, Proteins and Oligonucleotides.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L.K. Pannell	Visiting Scientist	LAC, NIDDK
P. Lecchi	Visiting Fellow	LAC, NIDDK
H. Le	Stay-in-school	LAC, NIDDK
Q-l. Pu	Special Volunteer	LAC, NIDDK
U. Rajamani	Special Volunteer	LAC, NIDDK

COOPERATING UNITS *(if any)*

Daly and Jerina, LBC, NIDDK; Fales, LBC, NHLBI; Basile, LNS, NIDDK; Gustafson, Beutler and Cardellina, NCI, FCRDC; Miles, LMB, NIDDK; Wingfield, OIR,OD; Byrd and Baldeserri, ABL, FCRDC; Peterkofsky, IR, NHLBI; Kashman, Tel Aviv Univ, Israel.

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Structural Mass Spectrometry Unit, Instrumentation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Specialized mass spectrometry analyses are provided to the laboratory and to other collaborating units. The emphasis of this work is split between identification of trace organic compounds isolated from biological sources and the mass spectrometry of biopolymers (e.g. peptides, proteins and oligonucleotides). A very close working relationship and collaboration is maintained with the Laboratory of Biophysical Chemistry, NHLBI, especially with the research into, and use of techniques for, mass spectrometry of macromolecules. Approval has been received to upgrade the electrospray source on the JEOL SX102 instrument to enhance stability and sensitivity, and to facilitate interfacing to liquid chromatographic methods. This will be used as part of the Unit's effort into post-translational modifications of proteins. The capillary zone electrophoresis instrument continues to receive heavy usage from this laboratory and an investigator from BEIP. Use is made of the NHLBI laser desorption mass spectrometer for the analysis of proteins, protein digests and oligonucleotides as part of our growing work in biopolymer mass spectrometry. Using a combination of this instrument and the JEOL mass spectrometer, methods have been developed and applied for the determination and identification of disulfide bonds in proteins, and for the analysis of oligonucleotides and DNA by mass spectrometry. In the small molecule area, collaborative interest has continued in the identification of biologically active natural products, especially those of interest in AIDS research and treatment. Samples analyzed derive from many facilities and researchers and approximately 2600 samples were analyzed on the JEOL and over 2000 on the MALDI instrument.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 58501-09 LN

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors for Neurotransmitters & Drugs in Brain & Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: P. Skolnick, Ph.D., Chief, LN; Pharmacologist: A. S. Basile, Ph.D.; Staff Fellow: L. Fossom, Ph.D.; PRAT Fellow: R. Hu, M.D.; Visiting Fellows: Elena Kustova, Ph.D.; P. Popik, M.D., Ph.D.; L-M Zhou, Ph.D.; Visiting Associates: Y. Li, M.D.; Y. Sei, M.D., Ph.D.; IRTA Fellows: N. Huang, M.D., Ph.D.; C. Kaufman, Ph.D.; R.T. Layer, Ph.D.; D. Gunnerson, Ph.D.; Predoctoral IRTAS: T. Lyon, B.S.; Special Volunteers: K. Bambha, M.S.; J. Hee-Ha, M.D., Ph.D.; Guest Workers: M. Beneveniste, Ph.D.; E. Moody, M.D.

COOPERATING UNITS *(if any)*

K. Rice, LMC, D. von Lubitz, LBC, NIDDK; J. Witkin, ARC, NIDA; M. Heyes, LCS, NIMH; J. Hartley, H. Morse, LI, NIAID; R.C. Drugan, Brown University; A. Lewin, RTI; continued on separate sheet

LAB/BRANCH
Laboratory of Neuroscience

SECTION

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 13.0	PROFESSIONAL: 13.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High affinity, stereospecific recognition sites (receptors) for neurotransmitter, neuromodulators, and many clinically useful drugs have been identified in both peripheral tissues and the central nervous system. The interaction of a neurotransmitter, neuromodulator or a drug with a specific recognition site initiates a series of events (for example, the opening of an ion channel or activation of an enzyme) resulting in either a physiological response (in the case of a neurotransmitter or neuromodulator) or a pharmacological effect (in the case of a drug). Furthermore, the presence of recognition sites for synthetic substances indicates that endogenous substances may also be present which can mimic (or antagonize) the effects of exogenously applied (synthetic) compounds. Studies are in progress to characterize "recognition-effector" systems with special emphasis on ligand (transmitter)-gated ion effector systems, to isolate and identify novel endogenous ligands of physiological and pathophysiological importance, to develop appropriate model systems to examine these phenomena, and to relate recognition-effector systems to both physiological and pathophysiological processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 59,501-09 LMC

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Design and Synthesis of Drugs Acting on Central and Peripheral Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. C. Rice	Chief, Drug Design & Synth. Section	LMC-NIDDK
Co PI:	A. E. Jacobson	Deputy Chief, LMC	LMC-NIDDK

Others: P. Fleming, J. Pinto, C. Bertha, D. Lewis, F.H. Wojnicki, Y. Zhang, IRTA; S. Calderon, D. Matecka, Visiting Fellows; J. Glowa, Expert. All LMC, NIDDK.

COOPERATING UNITS (if any)
LN-NIDDK (P. Skolnick); U Arizona (F. Porreca); U of Michigan (J. Woods, C.P. France); CC-NM (D. Kiesewetter, M. Channing); U Alabama (E. Blalock); BPB-NIMH (A. Pert); Research Triangle Institute (F.I. Carroll); ARC, NIDA (J.L. Katz, R.B. Rothman, H.C. Akunne); NCI (T.R. Burke Jr.).

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION
Drug Design and Synthesis Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 9.5	PROFESSIONAL: 9.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The abuse of cocaine is widely recognized to be an extremely serious health and social problem of epidemic proportions for which there is no effective treatment. This problem continues to be exacerbated by cocaine base ("crack") smoking, an extremely reinforcing and dependence producing route of self-administration. Our approach to treatment and prevention of cocaine abuse involves development of high affinity, slowly dissociating, low intrinsic activity cocaine receptor agonists. This approach has been validated with our finding that the lead compound GBR 12909 prevents cocaine self-administration in rhesus monkeys trained to self-administer cocaine with no effect on normal behavior as measured by food maintained responding. Studies with repeated administration of drugs from our chemical synthesis program and GBR 12909 have shown sustained therapeutic effects on cocaine self administration. For example, in a 12 day treatment study with GBR 12909, cocaine self-administration was eliminated in monkeys with no effect on food intake. No evidence for development of tolerance (diminishing activity of the treatment drug) was observed. When the treatment drug was discontinued after 12 days, cocaine self-administration returned to the pretreatment level but could again be eliminated by renewed GBR 12909 treatment. Food-maintained responding was used as a "control" for unwanted, behaviorally-impairing, side effects of test agents. These selective effects were unlike those of *d*-amphetamine or long-acting cocaine analogs (e.g. CFT) suggesting that selective GBR 12909 and related DA reuptake inhibitors do not simply substitute for the properties of cocaine responsible for its abuse potential. Taken together, our studies indicate GBR 12909 as well as drugs synthesized in our program with this profile are potentially valuable agents for the treatment and prevention of human cocaine abuse.

Opiate receptors play major roles in analgesic and euphoric effects of opiate drugs. Recent cloning of cDNAs encoding the rodent and human mu receptor have opened up new opportunities for research in opioid pharmacology and revealed high homology between the predicted receptors but also some sequence differences. Our ligand selectivity profiles of the human and rat mu receptors revealed modest differences in affinities for morphine and naloxone in COS but not CHO cells. Interesting differences between these data and data previously obtained with the peptide agonist [3H]DAMGO suggest that the peptide and alkaloid agonists may label different domains of the mu receptor.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-854-809

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 59,502-07 LMC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design, Synthesis and Evaluation of Medicinal Agents and Research Tools

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.E. Jacobson	Deputy Chief, LMC	LMC-NIDDK
Co PI:	K.C. Rice	Chief, Drug Design & Synth. Sect.	LMC-NIDDK
Others:	C. Bertha, D. B. Lewis	IRTA	LMC-NIDDK
	M. Mattson	Biologist	LMC-NIDDK
	D. Matecka	Visiting Fellow	LMC-NIDDK
	K. Homma	Special Volunteer	LMC-NIDDK

COOPERATING UNITS (if any)

Naval Research Lab (C. George); ARC-NIDA (R.B. Rothman); Medical College of Virginia, VCU (E.L. May); University of Michigan (J. Woods); University of Mississippi Medical School (W. Woolverton).

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.75

PROFESSIONAL: 2.75

OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ligands for the sigma receptor system were studied. Sigma receptors are non-dopaminergic, non-opioid receptors which bind antipsychotic drugs and have been implicated in neural regulation of motor behavior and modulation of transmitter release upon electrical stimulation of smooth muscle preparations. A considerable variety of compounds, of very different structural types, can interact with the known sigma receptor subtypes. Various disubstituted piperazines have been synthesized and found to have good affinity for the sigma-1 receptor. However, zipeprol, which is also a disubstituted piperazine (4-(2-methoxy-2-phenylethyl)-alpha-(methoxyphenylmethyl-1-piperazineethanol), is a potent sigma-2 receptor agonist and is somewhat selective for that receptor subtype (sigma-1/sigma-2 ratio ~7). Zipeprol has been found to have a novel spectrum of action involving both opioid and non-opioid effects, as well as its effects due to its interaction with the sigma receptor.

In our continuing study of the opioid receptor system, we have examined conformationally fixed derivatives of the potent class of narcotic agonists, 5-(3-hydroxyphenyl)morphans. This class of opioid is known to exhibit potent antinociceptive activity as well as narcotic agonist-antagonist activity, depending on the particular optical isomer. The parent compounds have a rigid 2-azabicyclo[3.3.1] nonane ring sysem with a freely rotating phenyl group attached at the 5-position. In order to gain insight into the topological features of opioid receptor binding sites we have undertaken a study aimed at determining the optimum torsion angle between the phenyl ring and piperidine ring for binding to the mu opioid receptor. There are a total of 8 isomers possible in this class of oxide-bridged molecules, of which six have been successfully synthesized in our laboratory, thus far. The synthesis of a seventh is now being approached through a key intermediate in the postulated synthesis scheme.

The synthesis of chiral GBR 12909 analogs with trans-2,5-dimethyl substitution on the central piperazine ring in our laboratory has produced high affinity, slowly dissociating, low intrinsic activity cocaine receptor agonists which may prove useful as treatment agents. A new methodology has been devised towards the synthesis of a large variety of chiral di- and mono-substituted GBR analogs. This new enantioselective synthetic methodology involves the use of enantiomerically pure amino acids.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 59602-21 LMC

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interferon Induction and Action. The Antiviral Action of Nucleoside Analogues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. F. Torrence	Section Chief	LMC/NIDDK
Others:	M. Player	IRTA	LMC/NIDDK
	W. Xiao	Visiting Fellow	LMC/NIDDK
	R. Zhang	Visiting Fellow	LMC/NIDDK

COOPERATING UNITS (if any)
Dr. R. Silverman, Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH
Dr. L. Beigelman, Ribozyme Pharmaceuticals Inc., Boulder, CO

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Biomedical Chemistry

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 4.0
PROFESSIONAL: 4.0
OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interferon-induced enzyme activities such as the 2-5A synthetase, the 2',5'-phosphodiesterase, and the 2-5A-dependent ribonuclease are studied with the goal of understanding their role in the action of interferon, the induction of interferon by dsRNA, and the control of cell growth and differentiation. Analogues of a mediator of interferon action are synthesized in order to define the relationship between oligonucleotide structure and binding to and activation of the 2-5A-dependent endonuclease. The eventual goal is to understand the biological role of the 2-5A system and to explore the potential of exploitation of this system in chemotherapy. Finally, a number of new approaches to pharmacologically active nucleoside analogues are pursued.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-854-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 59-701-22 LMC

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Reactions and Immunochemistry of Carbohydrates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Cornelis P. J. Glaudemans	Section Chief, SOC	LMC/NIDDK
Others:	P. Kovac	Research Chemist	LMC/NIDDK
	E. M. Nashed; E. Petráková	Sr. Staff Fellow; Visiting Assoc.	LMC/NIDDK
	L. Mulard; L. Ping-Shen	Visiting Fellows	LMC/NIDDK
	C. E. Miller; Y. Ogawa	IRTA, Special Volunteer	LMC/NIDDK

COOPERATING UNITS (if any)

J. B. Robbins, NICHD
R. Schneerson, NICHD

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Section on Carbohydrates

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 7.0	PROFESSIONAL: 7.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section works on elucidating the interaction of (complex) carbohydrate immuno-determinants with monoclonal antibodies (MAbs). Such interactions pertain to all ligand-protein interactions. We are executing:

1. Physico-chemical studies on antibody/antigen systems (*Sh. dys.* type 1, *V. cholera*).
2. The synthesis of ligands mimicking immunodeterminants of pathogenic entero-bacteria for affinity studies.
3. The study of immunodeterminants of these bacteria, so as to evaluate procedures for conjugate-vaccine development.
4. The amino acid sequencing by cDNA technique of monoclonal immunoglobulins obtained against pathogenic bacteria, and studies on the interaction of immunodeterminants with their energy minimized, tertiary structures.

We are continuing to determine the specific interaction between microbial polysaccharides, and a number of monoclonal antibodies, such as *Shigela dysenteriae* type 1,*Vibrio cholera* and its monoclonal antibodies, and have prepared ligands to probe the fundamental nature of these antibody-antigen association.
We have prepared additional complex fragments of the capsular polysaccharide of *Shigella dysenteriae* type 1 by sophisticated syntheses. These include deoxy and deoxyfluoro derivatives of the determinant. Studies are continuing on specific interactions between ligands and the binding area of a monoclonal antibodies specific for this disease-causing micro-organism. The amino acid sequence of some of these antibodies have been determined by cDNA cloning. Three dimensional antibody structures have been evaluated to delineate actual molecular forces involved in the binding.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 59801-04 LMC

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Evaluation of Potential Cocaine Antagonists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.R. Glowa	Expert, Behavioral Pharmacol. Unit	LMC/NIDDK
Others:	Frank Wojnicki	IRTA	LMC/NIDDK
	Gabrielle Bonhomme	summer IRTA	LMC/NIDDK
	Gregory Charney	Howard Hughes student	LMC/NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary mission of the Behavioral Pharmacology Unit is to assess the behavioral effects of pharmacological agents, designed by the LMC and other sources, for their efficacies to modify the abuse potential of cocaine. The usual means by which this mission is accomplished is the direct assessment of these agents on drug-seeking behavior of monkeys. Twenty four monkeys have been surgically prepared, and now undergo daily testing. At least twenty compounds have been assessed, some in considerable detail to validate the system in comparison to other methods. Several papers have been presented at international meetings and publications regarding our findings and methods are now in print and in press as we continue to assess agents of interest. The laboratory has developed several additional capabilities to facilitate the understanding of relevant end points of drugs of abuse related to abuse potential. For example, neuroendocrine and immune function, as well as other behavioral end points, can be evaluated initially in rodent models. The ability to PET scan monkeys that have acquired self-administration behavior is being explored. We have established collaborative efforts with the local neuroscience community to provide additional support, primarily using behavioral genetics as a tool. In addition, we have established behavioral assays for additional ligand-behavior interactions which may be relevant to unique effects of drugs of abuse, developed methods to determine undesirable side effects (behavioral toxicology, and improved methods to quantify potential risks of those adverse effects (risk assessment).

U.S. GOVERNMENT PRINTING OFFICE 1991 0-654-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 59801-04 LMC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Potential Cocaine Antagonists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	J.R. Glowa	Expert, Behavioral Pharmacol. Unit	LMC/NIDDK
Others:	Frank Wojnicki	IRTA	LMC/NIDDK
	Gabrielle Bonhomme	summer IRTA	LMC/NIDDK
	Gregory Charney	Howard Hughes student	LMC/NIDDK

COOPERATING UNITS (If any)

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis Section

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary mission of the Behavioral Pharmacology Unit is to assess the behavioral effects of pharmacological agents, designed by the LMC and other sources, for their efficacies to modify the abuse potential of cocaine. The usual means by which this mission is accomplished is the direct assessment of these agents on drug-seeking behavior of monkeys. Twenty four monkeys have been surgically prepared, and now undergo daily testing. At least twenty compounds have been assessed, some in considerable detail to validate the system in comparison to other methods. Several papers have been presented at international meetings and publications regarding our findings and methods are now in print and in press as we continue to assess agents of interest. The laboratory has developed several additional capabilities to facilitate the understanding of relevant end points of drugs of abuse related to abuse potential. For example, neuroendocrine and immune function, as well as other behavioral end points, can be evaluated initially in rodent models. The ability to PET scan monkeys that have acquired self-administration behavior is being explored. We have established collaborative efforts with the local neuroscience community to provide additional support, primarily using behavioral genetics as a tool. In addition, we have established behavioral assays for additional ligand-behavior interactions which may be relevant to unique effects of drugs of abuse, developed methods to determine undesirable side effects (behavioral toxicology, and improved methods to quantify potential risks of those adverse effects (risk assessment).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 59-802-03 LMC

PERIOD COVERED
October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Elucidation of the Structure and Function of Sigma Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	W. D. Bowen	Res. Pharmacol., Chief, Receptor Biochemistry & Pharmacology Unit	LMC/NIDDK
Others:	B.J. Vilner	Visiting Scientist	LMC/NIDDK
	C. Torrence-Campbell; D. Joseph	IRTA	LMC/NIDDK
	W. Williams; D. Thomassen	Microbiologist; Biologist	LMC/NIDDK
	A. Eyssallenne; M. Ellis; F. Carpenter	MARC student; summer IRTA	LMC/NIDDK

COOPERATING UNITS (if any)
Research Triangle Institute (F.I. Carroll); U. California, Irvine (R. Matsumoto); George Washington University Medical Center (C.S. John).

LAB/BRANCH Laboratory of Medicinal Chemistry

SECTION Drug Design and Synthesis

INSTITUTE AND LOCATION NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 6.7 PROFESSIONAL: 4 OTHER: 2.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sigma receptors are saturable, high affinity binding sites for several important classes of psychotropic drugs including typical antipsychotic, antidepressant, anticonvulsant, and psychotomimetic compounds. They are likely to contribute to the beneficial and/or side-effect profile of these compounds. Our laboratory has developed several new ligands for these receptors, which include antagonists, sigma-2 selective compounds, and ligands with potential as imaging agents.

Characterization of two sigma antagonists with antidystonic effects in rats: The novel sigma ligands, N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD1047) and 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine (BD1063), were characterized in rats using binding assays and behavioral studies. In radioligand binding studies, the novel ligands showed marked selectivity for sigma binding sites, generally having a 100-fold or better affinity for sigma sites compared to nine other tested receptors. BD1047 and BD1063 had no effects on their own when unilaterally microinjected into the red nucleus of rats, but both compounds attenuated the dystonia produced by di-o-tolylguanidine (DTG) and haloperidol. Both ligands shifted the dose curve for DTG to the right. BD1047 and BD1063 appear to act as antagonists at sigma sites.

Ibogaine and its congeners are sigma-2 receptor-selective ligands with moderate affinity. Ibogaine exhibited moderate affinity for sigma-2 sites (Ki = 201 nM) and low affinity for sigma-1 sites (Ki = 8,554 nM), thus showing 43-fold selectivity for sigma-2 receptors. The congeners tabernanthine and racemic ibogamine had sigma-2 Ki = 194 nM and 137 nM, respectively. However, they showed 3- to 5-fold higher sigma-1 affinity compared to ibogaine, resulting in about 14-fold selectivity for sigma-2 sites over sigma-1. A potential ibogaine metabolite, O-des-methyl ibogaine, had markedly reduced sigma-2 affinity relative to ibogaine (Ki = 5,226 nM) and also lacked significant affinity for sigma-1 sites. Thus sigma-2 receptors may play a role in the actions of ibogaine.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 60000-01 LCBB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Nuclear Envelope in Intracellular Protein Sorting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.A. Hanover	Research Chemist	LCBB, NIDDK
Others: M. Miller	IRTA	LCBB, NIDDK
S. Bailer	Visiting Fellow	LCBB, NIDDK
W. Lubas	Research Associate	LCBB, NIDDK
T. Sweitzer	IRTA	LCBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biochemistry and Biology

SECTION

Cell Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nuclear transport plays a key role in regulating the levels and activities of transcription factors, nuclear kinases, steroid hormone receptors and DNA replication factors. The nuclear pore complex (NPC) mediates the transport of mRNA and proteins across the nuclear envelope. Previously, we described a family of phosphorylated glycoproteins which are components of the NPC. Interestingly, changes in the degree of phosphorylation and glycosylation occur during the cell cycle that may be associated with assembly and disassembly of nuclear pores. The major nuclear pore protein, p62 has been studied in greater detail. In addition, the enzyme which mediates O-linked GlcNAc addition to p62 has been purified and microsequenced. A homolog of the O-linked GlcNAc transferase has been identified in *Caenorhabditis elegans* which may allow genetic manipulation of the enzyme in an intact organism.

Using digitonin permeabilized cultured cells, we have studied nuclear and nucleolar transport of fluorescent conjugates bearing the appropriate localization signal. Such conjugates concentrated in the nucleus and nucleolus, respectively, when supplied with a cytosolic fraction and ATP. Nuclear Transport requires both Ca+2 and GTP suggesting that nuclear transport may be coordinately regulated with other signal transduction pathways during cellular activation. We have extended these studies using patch-clamp electrophysiology to examine regulated ion flow through the NE of cardiac myocytes. We detected large conductance ion channel activity at the NE resembling that of gap junction channels. Activation of these channels required cytosolic factors. The channels were blocked by GTP-γ-S, wheat germ agglutinin and nuclear pore-specific antibodies. Thus the large conductance nuclear ion channel activity is likely to be intrinsic to nuclear pores. Using recombinant TATA-binding protein, c-Jun, NF-κB and SP1 it was also possible to demonstrate transcription factor translocation through the nuclear pore channel by patch-clamp measurements and atomic force microscopy. These observations strongly support the notion of a nuclear pore channel that can be measured using elecrophysiological means. Since these measurements can be made in real-time it should be possible to examine conformational alterations in the nuclear pore during transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 60001-01 LCBB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Intracellular Traffic in HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John A. Hanover	Research Chemist	LCBB, NIDDK
Others:	David W. Frank	IRTA	LCBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biochemistry and Biology

SECTION

Cell Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transport across the nuclear membrane is required at numerous steps in the life cycle of the human immunodeficiency virus (HIV) and other retroviruses. Once in the cytoplasm, HIV RNA is reverse-transcribed into double-stranded DNA which then must enter the nucleus. This is the preintegration complex which contains DNA, RNA, reverse transcriptase integrase and matrix protein. A growing amount of evidence points to the viral matrix protein of HIV as a mediator of the nuclear transport event. Efforts are underway to determine how the matrix protein may be involved in nuclear transport of the DNA-RNA-protein complex. In recent studies, the matrix protein has been isolated in recombinant form and its nuclear localization examined. Other retroviral regulatory proteins also enter the nucleus to perform their function, and viral transcripts are exported to the cytoplasm. The viral proteins, *rev* and *tat*, have been identified as key regulators of the transcription and transport of HIV envelope mRNA out of the nucleus. These proteins contain sequences that are necessary and sufficient for targeting to the nucleolus. An *in vitro* system using permeabilized cells has been developed to look at the nucleolar targeting of proteins bearing such targeting signals. We have observed that transport to the nucleolus is an active process. Recently, it has been demonstrated that a reduction in cellular GTP levels induced by inhibitors of IMP-dehydrogenase is correlated with a reduction in the nucleolar accumulation of other proteins. We have found that these inhibitors also block the nucleolar targeting of HIV *rev* protein. However, these inhibitors, which include mycophenolic acid, did not affect nucleolar accumulation of the model protein in permeabilized cells. Moreover, 100-fold reduction of GTP levels in the *in vitro* assay did not affect nucleolar accumulation of the model protein. Efforts are underway to identify cytosolic components required for nucleolar transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 60003-01 LCBB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ultrastructural Immunocytochemistry of Lipid Metabolism in Cells and Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Joan Blanchette-Mackie	Research Biologist	LCBB, NIDDK
Others:	Robert O. Scow	Chief, Endocrinology Sect.	LCBB, NIDDK
	Nancy K. Dwyer	Biologist	LCBB, NIDDK
	Edward Neufeld	IRTA, Post-Doctoral Fellow	LCBB, NIDDK
	Therese Barber	IRTA, Pre-Doctoral Fellow	LCBB, NIDDK
	Hailan Tang	Special Volunteer	LCBB, NIDDK

COOPERATING UNITS (if any)

Dr. Peter Pentchev. Dev Metab Neurol Branch, NINCDS, NIH; Dr. Constantine Londos, Lab Cell Develop Biol, NIDDK, NIH; Dr. Richard E. Pagano, Mayo Clinic, Rochester, MN; Dr. R.M. Evans, University of Colorado, Denver, CO.

LAB/BRANCH

Laboratory of Cell Biochemistry and Biology

SECTION

Lipid Cell Biology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Perturbations in intracellular cholesterol transport result in loss of homeostatic responses that regulate cholesterol synthesis, uptake and esterification. The role of the plasma membrane cholesterol pool (comprising 90% of total cellular cholesterol) on intracellular cholesterol transport was examined in living cultured cells derived from normal patients and those with Niemann Pick type-C (NP-C) disease, a genetic lesion of cholesterol metabolism. Treatment of fibroblasts with hydroxypropyl-b-cyclodextran, selectively removes plasma membrane cholesterol. ^{3}H-cholesterol resident at the plasma membrane and derived by *de novo* synthesis from ^{3}H-acetate was removed from normal and NPC human fibroblasts with similar kinetics. In contrast, arrival of exogenously derived LDL-^{3}H cholesterol, to the plasma membrane from lysosome was slower in NP-C compared to normal fibroblasts. LDL-cholesterol passes through this pathway is impaired in NP-C cells. 58035, a drug that inhibits ACAT in the endoplasmic reticulum (ER), causes accumulation of LDL-cholesterol in the ER. Although 58035 blocks ACAT, the drug enhances other homeostatic responses whereas, the NP-C mutation inhibits all homeostatic responses. Thus, exogenously derived cholesterol that reaches the ER can signal these cellular responses but cholesterol that accumulates in the proximal, lysosomal-Golgi segment of the pathway can not.

The key enzyme in cellular lipolysis, hormone-sensitive lipase (HSL) was present on the surface monolayer of most triacylglycerol containing lipid droplets in adipocytes and steroidogenic cells stimulated with isoproterenol, whereas little HSL was associated with the surface of lipid droplets in unstimulated cells. In contrast, perilipin, which resides on the surface of lipid droplets in unstimulated cells, appears to disperse from that site in stimulated cells. Thus, the hormone induced increased access of intracellular HSL to its triacylglycerol and cholesterol ester substrates may be modulated by translocation of perilipin from the lipid droplet surface. Lipoprotein lipase (LPL), an enzyme required for lipolysis of triacylglycerol in circulating lipoprotein, is made by parenchymal cells and transported to capillaries. Parenchymal cells, derived from combined lipase deficient (cld/cld) mice make an inactive form of LPL. Our studies on cultured brown adipocytes derived from normal and (cld/cld) newborn mice show that mutant cells cannot transport LPL from site of synthesis in ER to Golgi for processing to an active lipase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-60050-01 LCBB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vitamin D Resistance and Related Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Barsony, M.D.	Visiting Scientist	LCBB, NIDDK
Others:	W. McKoy	Chemist	LCBB, NIDDK
	I. Renyi, Ph.D.	Visiting Associate	LCBB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biochemistry and Biology

SECTION

Cell Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

With recognition that vitamin D is the precursor for 1,25-dihydroxyvitamin D, it has become possible to characterize defects in the target actions of 1,25-dihydroxyvitamin D. We have demonstrated a broad spectrum of manifestations of hereditary resistance to 1,25(OH)2D, ranging from infantile rickets with alopecia and no intestinal response to calciferols to adult onset osteomalacia with satisfactory intestinal response to high doses of calciferols and with no epidermal abnormalities. This syndrome usually results from a mutation in the gene for the vitamin D receptor. Skin fibroblasts from all subjects with hereditary resistance to 1,25(OH)2D display abnormalities in this effector system, and defects in many discrete steps of this pathway have been identified with these cells. Cells with mutations in the 1,25(OH)2D effector pathway can be used to explore mechanisms of calciferol action. They have been used to establish that the 1,25(OH)2D receptor mediates an extremely rapid (1-3 minutes) rise of cyclic GMP in response to 1,25(OH)2D3 and that certain receptor mutations compromise many receptor functions but allow another function to be retained normally. This establishes that 1,25(OH)2D receptors couple to different responses by distinct mechanisms. With the development of a new, pharmacologically relevant fluorescent 1,25(OH)2D, it became possible to study vitamin D receptor activation in living cells and recognize abnormalities in vitamin D receptor translocation from the cytoplasm to the nucleus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69000-30 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK
	M. de Courten	Visiting Fellow	DAES, NIDDK
	R. Fernandes	Visiting Fellow	DAES, NIDDK
	J.L. Roumain	Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4	2.2	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to identify the determinants of non-insulin-dependent diabetes (NIDDM), various types of arthritis, and gallbladder disease, and elucidate the natural history of the diseases. Genetic and environmental risk factors for NIDDM have been studied in the Pima Indians. The residents of the study area, approximately 5000 people, have participated in a longitudinal population study since 1965, allowing observations of the natural history of diabetes mellitus. Risk factors for obesity, hypertension, and cholelithiasis are also studied, along with the relationships of these diseases to diabetes and their effects on mortality rates. The genetics of diabetes is studied by means of family studies and relationships of genetic markers to disease. The roles of obesity, serum insulin concentrations, impaired glucose tolerance, occupational and leisure-time physical activity and diabetes in relatives are assessed.

The age-specific incidence rates of NIDDM have been increasing among the Pima Indians. Analyzed by birth cohort, age-sex-adjusted incidence rates have increased by an average of 13% in each successive decade of birth year, suggesting that exposure to diabetogenic lifestyle factors may have increased progressively over the 80-year span of birth cohorts examined.

Follow-up for development of diabetes after measurement of body size by a variety of methods indicated that several methods were equivalent as predictors. Thus simple measures such as waist circumference, weight, and body mass index may be just as useful as more complicated measures such as estimated percent body fat by bioelectrical impedance.

The association of diabetic nephropathy and diabetes was studied in Pima Indian pedigrees. The presence of nephropathy in a parent with diabetes relative to diabetes alone was associated with 2.5 times the odds of diabetes in the offspring. These results are compatible with the hypothesis that the susceptibility to renal disease in the parents and to diabetes in the offspring are due to shared familial environmental factors or the same gene or set of genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69001-26 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications and Outcome of Diabetic and Prediabetic Pregnancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Biostatistics and Data Management Section, PECRB

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the project are to determine the effects of abnormal glucose tolerance on outcome of the pregnancy, to determine long term prognosis for the women and their offspring, and to identify diabetes and impaired glucose tolerance during pregnancy in women in the Gila River Indian Community. By means of a glucose tolerance test as well as chart review, the diabetes status of every woman is determined at two-yearly intervals and during the third trimester of each pregnancy. Offspring are followed from age 5 years and the effects of the diabetic intrauterine envrionment are evaluated in conjunction with other risk factors for obesity and diabetes.

Pima Indians who had been breast fed as infants were found to have lower rates of non-insulin-dependent diabetes than those who had not been breast fed. To explore other possible consequences of this breast feeding, the effect of having been breast fed in infancy on glucose concentration during pregnancy was examined. Glucose concentration was measured after a 75g oral glucose load in 93 Pima women aged < 25 years who, as infants, had been breast fed for ≥ 1 month and 88 who had not. When controlled for other factors influencing glucose tolerance (age, BMI, birth weight, diabetes in ≥ 1 parent, and maternal diabetes during pregnancy), the mean 2-h plasma glucose concentration was lower in pregnant women who had been breast fed (5.7 mmol/l vs 6.3 mmol/l, $p=0.034$). Thus, breast feeding, which is associated with less diabetes in nonpregnant Pima Indians, is also associated with lower plasma glucose concentrations during pregnancy.

Seventeen of 73 women who were pregnant and 114 of 244 women who were not pregnant when IGT was recognized developed diabetes within 10 years. Controlled for glucose concentration, age, body mass index, parity and duration of follow-up, those who were not pregnant were at higher risk of diabetes than those who were pregnant (odds ratio=1.71, 95% confidence interval=1.01-2.91). This study suggests that, during pregnancy, although women with IGT are at a higher risk of diabetes than women with normal glucose tolerance, they are at lower risk than non-pregnant women with a similar glucose concentration who, in the clinical setting, are likely to remain unrecognized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69006-25 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gila River Indian Community Autopsy and Mortality Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	R.G. Nelson	Special Expert	PECRB, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	M.L. Sievers	Guest Researcher	DAES, NIDDK
	R.L. Hanson	Epidemiology Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The causes of death and postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects. Medical records are reviewed for the determination of cause of death and for the occurrence of certain serious diseases or complications of diabetes.

The purpose of the study is to relate the outcome and cause of death to events or risk factors measured in life among Pima Indian residents of the Gila River Indian Community, particularly in relation to diabetes, cardiovascular diseases and gallbladder disease. Death Certificates are obtained on all members of the Gila River Indian Community In addition, post mortem examinations and all available medical records pertaining to the subjects are obtained to ascertain conditions present at the time of death and ascertain cause of death as precisely as possible. These records are reviewed in a standardized way for evidence of the complications of diabetes, vascular disease, neoplasms and other conditions. The records are used to determine the causes of death and incidence of complications associated with diabetes and other conditions identified initially during life by the longitudinal epidemiologic studies in the population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69009-30 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Arthritis and Rheumatism in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	S.R. Pillemer	Medical Officer	OPECA, NIAMS
	R. Fernandes	Visiting Fellow	DAES, NIDDK
	R. Hirsh	IRTA	OPECA, NIAMS

COOPERATING UNITS *(if any)*

Indian Health Service; NIAMS

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of osteoarthrosis, rheumatoid arthritis and ankylosing spondylitis are being determined by means of clinical, radiographic and serological examinations carried out prospectively at two-yearly intervals among adults of the Gila River Indian Community (Pima Indians) in Arizona, in conjunction with epidemiological studies of diabetes in the same community. The purpose of this investigation is to ascertain the determinants of these diseases in the population, and to identify factors which predispose to or alter the natural history of progression of the disease. Host factors such as age, sex, and various gene markers including HLA and Gm, together with various potential environmental determinants, such as obesity and evidence of exposure to infectious agents, are being investigated prospectively to determine their relationship to the development of these diseases. Longitudinal data have now been collected for 25 years and represent a unique data set for epidemiological studies of arthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69015-13 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-Sectional and Longitudinal Study of "pre-diabetes" in the Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	D. Mott	Supervisory Res. Chem.	IHS
	R. Pratley	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indians of Arizona have the highest reported prevalence and incidence of non-insulin dependent diabetes mellitus (NIDDM) of any population in the world. Beginning in 1982, our section has studied a subset of this population to determine the etiologic factors that predispose non-diabetic individuals to develop the disease. Subjects are admitted yearly to the clinical research ward to undergo body composition analysis, an oral glucose tolerance test, an intravenous glucose tolerance test, a standard mixed meal test, and a two-step hyperinsulinemic, euglycemic clamp to measure insulin action in vivo. Over 600 individuals have entered into the study and approximately 50 subjects have developed NIDDM. Obesity, central obesity, fasting hyperinsulinemia, and insulin resistance are each major risk factors for the developement of NIDDM. The acute insulin response to a intravenous glucose bolus is an additional, but weaker predictor. Since each of these factors are familial and predictive of diabetes, it is likely that the genetic determinants of these phenotypes contribute to the pathogenesis of NIDDM, which is known to be largely genetically determined. Thus, current effort on this project is directed to recruiting and studying the siblings of individuals who have previously participated in these studies. The phenotypic information generated from these studies will be used in conjunction with DNA markers in sib-pair linkage analyses to identify loci linked to insulin resistance, decreased insulin secretory function and obesity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69020-11 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin Resistance and the Regulation of Muscle Glycogen Synthase Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	D. Mott	Supervisory Res. Chem.	IHS
	P. Permana	IRTA	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are currently characterizing the abnormalities for regulation of human muscle glycogen synthesis in insulin-resistant subjects. In insulin-resistant subjects, fasting glycogen synthase phosphatase (PP-1) activities are reduced and fail to show the peak insulin stimulation observed for insulin-sensitive subjects at 10-20 minutes. Phosphorylation of the G-subunit bound to the PP-1 catalytic subunit in the GM fraction regulates PP-1 activity. Western blots of muscle extracts indicate that the G-subunit has increased immunoreactivity in insulin resistant subjects. Glycogen synthase kinase-3 (GSK3A) and inhibitor-2 (IPP2) have been studied as candidate genes because the kinase regulates IPP2 which can inactivate the phosphatase responsible for activation of glycogen synthase and insulin mediated glucose storage. GSK3A has been localized to 19p13 and partially characterized for intron/exon boundries and structural identity with rat GSK3A. Two dinucleotide microsatellite markers from this loci have been sequenced and used to type DNA from 900 Pima Indians. Marker data will be used to determine possible linkage or association of this loci with insulin resistance and obesity. IPP2 has been localized to 3q29. A psuedogene lacking intron/exon boundries has been identified on chromosome 5. Intron/exon boundries have been identified for a total of 6 exons. Sequence analysis on 17 insulin resistant and 4 insulin sensitive subjects indicates no abnormalities in exons 2, 3 and 4.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69024-09 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

WHO Collaborating Center for Epidemiologic and Clinical Investigations in Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECR, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

World Health Organization, Non-Communicable Diseases Program, Geneva, Switzerland, (Foreign), World Health Organization Collaborating Centers for Diabetes (Foreign), China-Japanese Friendship Hospital, Beijing, China (Pan Xiaoren) (Foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Phoenix Epidemiology and Clinical Research Branch was designated as the WHO Collaborating Center for Design, Methodology and Analysis of Epidemiological and Clinical Investigations in Diabetes in 1986. The purposes of the Center are to collaborate with the World Health Organization in the implementation of the WHO/IDF action program to provide advice, consultation and collaboration with other investigators in the design, methodology and analysis of epidemiology and clinical diabetes (NIDDM) and its complications. The center assists in the development and application of standardized methods for epidemiological and clinical investigations and data analysis relating to the etiology and pathogenesis of non-insulin-dependent diabetes (NIDDM) and its complications. The center will collaborate with those interested in applying such techniques elsewhere. The Center will advise and help in the design of new studies, including on site assistance when necessary. The Center serves to train investigators from many parts of the world in diabetes epidemiology and clinical research.

The Center participates in the WHO Multicenter Study of Vascular Disease in Diabetes, which is examining the mortality and incidence of vascular complications of diabetes among different ethnic groups in different countries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69028-07 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	P.H. Bennett	Chief	PECRB, NIDDK
	C. Bogardus	Chief	CDNS, NIDDK
	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

NIAAA; Bowman-Gray Medical School, Winston-Salem, NC; Indiana University, Indianapolis, IN; Johns Hopkins University, Baltimore, MD; Cedars Sinai Medical Center, Los Angeles, CA.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	0.7	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Non-insulin dependent diabetes mellitus is a common chronic disease that develops in most populations in late middle age. The Pima Indians of Arizona have the highest reported prevalence of this disease in the world and in contrast to many populations the disease often presents at an earlier age. As a result of long-term epidemiological studies in the total population, the familial nature of the disease has been well documented, and segregation analyses suggest the possibility of inheritance by a single additive major gene.

This project will search for genetic determinants of NIDDM using the techniques of genetic linkage analyses with genetic markers to identify the chromosomal location of inherited determinants of NIDDM in the Pima Indian population. A number of informative pedigrees have been identified and lymphoblast cell lines from informative members of these pedigrees established. DNA from these lymphoblasts is isolated and polymorphic probes applied to search for evidence of linkage of these markers and NIDDM. Probes with established chromosomal locations are being used to screen the genome to detect genetic linkage with NIDDM as described in Project# Z01 DK 69055-02 (Linkage of Markers on chromosome 7 with NIDDM in Pima Indians).

Searches for mutations in mitochondrial DNA and in the IRS-1 gene revealed no variants which in other populations had been associated with diabetes. An allele in the ß3-adrenergic receptor gene is associated with earlier age of onset of diabetes among diabetic subjects, suggesting that this gene may play some role in susceptibility in NIDDM.

A refinement of the sib-pair linkage analysis method was developed to incorporate data on age of onset of affected siblings and age at examination of unaffected siblings. Simulation studies indicated that this method improves the power of sib-pair analyses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69030-07 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Contribution of Protein Tyrosine Phosphatase to Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	J. Rowles	IRTA	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; UTSWMC (M. Cobb)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously identified a novel PTPase in human skeletal muscle by PCR and reported the cloning of the full-length cDNA from a HeLa library. This PTPase is called PTP-PEST due to the presence of four regions rich in pro, glu/asp and ser/thr. To determine if the PEST sequences serve any regulatory role, the full length protein (PTP-PEST) and a truncated version that contains the entire catalytic domain but lacks all the PEST sequences (PTP-PESTdelta) have been expressed in Sf9 cells using a recombinant baculovirus. To determine if these PTPs may be involved in insulin signalling, they were coexpressed with a recombinant baculovirus that produces a soluble form of the insulin receptor (SIR), (kindly provided by Melanie Cobb, UTSWMC). Co-expression of PTP-PEST or PTP-PESTdelta with the SIR caused a reduction in the P-tyr content of SIR; this decrease correlated with a decrease (40-60%) in the tyrosine kinase activity of the SIR measured against an artificial substrate, PEY. We reported that the PTPase activities of both PTP-PEST and PTP-PESTdelta were also inhibited 40-60% when coexpressed with the SIR. However, co-expression of the parental baculovirus with PTP-PESTs has since shown variable results, ranging from no effect on enzyme activity, to an inhibition of the same magnitude as seen with the SIR. These results question the specifity of the effect seen with the SIR. We are in the process of expressing PTP-PEST in mammalian cells that can be treated with insulin to address to this problem in a more physiological manner. For this goal, the cDNA for both PTP-PEST and PTP-PESTdelta were modified at the 3 prime end by PCR to encode for a FLAG tag. This FLAG tag is a short amino acid sequence that is recognized by a monoclonal antibody capable of Western blotting and immunoprecipitating the expressed protein. (The peptide antibody that we obtained against PTP-PEST is only capable of immunoprecipitating denatured protein, thus greatly limiting its usefulness.) These modified cDNAs will be placed into a mammalian expression vector for transfection onto cells. Recently, the binding regions for SH3 domains have been determined for a number of proteins and several consensus sequences have emerged that are rich in proline residues. PTP-PEST contains several of these consensus sequences, suggesting that it may interact with SH3 domain containing proteins, for example, grb2. This hypothesis can be tested by immunoprecipitating PTP-PEST from mammalian cells and blotting with antibodies to SH3 domain containing proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69036-06 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiology of Complications of Non-Insulin-Dependent Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	M. de Courten	Visiting Fellow	DAES, NIDDK
	R. Fernandes	Visiting Fellow	DAES, NIDDK
	J.L. Roumain	Staff Fellow	DAES, NIDDK
	R.G. Nelson	Special Expert	PECRB, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.7	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to determine the incidence rates, rates of progression, and risk factors for the chronic complications of NIDDM. The study is conducted in the Pima Indians of the Gila River Indian Community, who have participated in a longitudinal epidemiologic study since 1965 (see project Z01 DK 69000).

Risk factors for the major complications of diabetes, retinopathy, nephropathy, coronary artery disease, and peripheral vascular disease are determined by longitudinal followup of diabetic subjects. Methods of ascertainment of these complications include fundus photography, measurement of urine albumin and serum creatinine concentrations, electrocardiography, and documentation of lower extremity amputations.

Some determinants of diabetic nephropathy precede the development of diabetes. An albumin-to-creatinine ratio (A/C) was measured in 237 nondiabetic Pima Indians without clinical proteinuria who developed diabetes at least one year later. After the onset of diabetes, the prevalence of an elevated A/C was directly related to the prediabetic A/C.

To determine if parental blood pressure was also associated with proteinuria in subjects with diabetes, the blood pressure was measured in both parents of 438 diabetic Pima Indians. The prevalence of proteinuria in the diabetic subjects if neither parent had hypertension was similar to that if one parent had hypertension, but was significantly higher if both parents had hypertension. This relationship remained when controlled for hypertension in the diabetic subjects.

Thus, prediabetic albuminuria and parental hypertension were risk factors for abnormal albumin excretion once a subject developed diabetes.

Medial arterial calcification (MAC) of the feet is another common complication of NIDDM, and its relationship with mortality was examined in 1363 diabetic Pima Indians. MAC was associated with mortality from all causes and from diabetic nephropathy. In an assessment of whether there was an effect on mortality which was in addition to that of proteinuria, either MAC or proteinuria alone was associated with a significant increase in risk and both MAC and proteinuria together conferred and even higher risk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69039-06 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dietary Survey of the Pima Indians of the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	V. Narayan	Visiting Scientist	DAES, NIDDK

COOPERATING UNITS *(if any)*

Cleveland Clinic Foundation, Phoenix, Arizona.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An age-sex-stratified sample of 600 residents of the Gila River Indian Community, ages 18-75 years, was recruited for a dietary survey. Dietary intake was estimated by the dietary history method to obtain quantitative food frequency information. Reported energy intake was higher in men and negatively related to age; thus, relationships with weight were analyzed by multiple regression controlling for sex and age. Energy intake in kilocalories (kcal) was positively associated with body weight or body mass index, adjusted for age and sex. Body weight was associated with absolute intake of the major dietary components, carbohydrate, fat, and protein, but not with any of these components expressed per 1000 kcal, suggesting that total energy intake, rather than proportions of specific components, was the variable having the strongest association with body weight. Alcohol consumption (in any amount) was higher in men and inversely associated with age and weight. Neither energy intake nor specific components were significantly associated with diabetes, after adjustment for age and sex, nor did diabetes affect the relationship between energy and weight, in a subset of 307 subjects (153 diabetics) examined for diabetes within one year of the diet history. The acceptance of obesity in this population may reduce the under-reporting of energy intake which has been postulated in other studies. Body weight in Pima Indians is weakly but positively associated with increased caloric intake.

In contrast to the findings from many other studies, reported calcium intake was positively related to blood pressure. Such associations are not uniform between populations, suggesting the importance of unmeasured confounding variables.

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DK 69040-06 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sodium-Lithium Countertransport and Blood Pressure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

University of Pittsburgh; University of Southern California

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0		0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Red blood cell sodium lithium countertransport is a genetic marker of hypertension in several ethnic groups. It reflects the sodium-hydrogen antiport activity in renal tubules. Several recent studies showed an association between sodium-lithium countertransport and predisposition to diabetic nephropathy. We are studying the relationships between sodium-lithium countertransport, nephropathy, and blood pressure in a sample of 200 Pima Indians.

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69045-04 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of the Intestinal Fatty Acid Binding Protein in Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	L. Baier	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Biochemistry, Albert Einstein Collge of Medicine, Bronx NY (J. Sacchettini); Department of Biophysics, Boston University, Boston, MA (J. Hamilton)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.10	1.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies identified a region on chromosome 4q that was linked to measures of insulin action. A candidate gene in this region is FABP2 which encodes the human intestinal fatty acid binding protein (IFABP). We identified a polymorphism in this gene which results in an alanine (Ala54) to threonine (Thr54) substitution at amino acid 54 of IFABP. We found a significant association between the Thr54-encoding IFABP genotype (frequency= 0.29) and increased fasting lipid oxidation rates and insulin resistance, and have further shown that recombinant Thr54 protein has a higher affinity for long-chain fatty acids as compared to recombinant Ala54 protein. To investigate physiologic consequences of the IFABP substitution, we analyzed fatty acid transport across cells expressing either Ala54 or Thr54 IFABP. Caco-2 cells provided a model system for expression of Thr54 and Ala54 IFABP since these cells functionally mimick polarized small intestinal epithelial cells, but do not endogenously express IFABP. We produced permanently transfected Caco-2 cell lines expressing equivalent amounts of Ala54 and Thr54 IFABP and compared their ability to transport long-chain fatty acids from their apical to basolateral surfaces. Transfected cell monolayers, grown on filter supports, were apically exposed to ^{3}H-long-chain fatty acids for 5 min-48 hours and then the basolateral media was analyzed for ^{3}H lipid that had been transported across the monolayer. At all time points, more ^{3}H lipid was transported across the Thr54-expressing cells as compared to the Ala54-expressing cells. The observed difference in transport of long-chain fatty acids across Thr54-expressing cells as compared to Ala54-expressing cells appears to be specific for molecules that interact with IFABP, since no difference in ^{14}C-glucose transport was observed across these cells. The nature of the basolaterally secreted ^{3}H-lipids components was also analyzed and we found that Thr54-expressing cells secreted a 4-6 fold greater amount of triglyceride to the basolateral media than Ala54 cells. A comparison of the protein structures of Thr54 and Ala54 IFABP is being solved by 3-D NMR in collaboration with J. Sacchettini and J. Hamilton.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69045-04 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of the Intestinal Fatty Acid Binding Protein in Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	L. Baier	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Biochemistry, Albert Einstein Collge of Medicine, Bronx NY (J. Sacchettini); Department of Biophysics, Boston University, Boston, MA (J. Hamilton)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.10	1.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies identified a region on chromosome 4q that was linked to measures of insulin action. A candidate gene in this region is FABP2 which encodes the human intestinal fatty acid binding protein (IFABP). We identified a polymorphism in this gene which results in an alanine (Ala54) to threonine (Thr54) substitution at amino acid 54 of IFABP. We found a significant association between the Thr54-encoding IFABP genotype (frequency= 0.29) and increased fasting lipid oxidation rates and insulin resistance, and have further shown that recombinant Thr54 protein has a higher affinity for long-chain fatty acids as compared to recombinant Ala54 protein. To investigate physiologic consequences of the IFABP substitution, we analyzed fatty acid transport across cells expressing either Ala54 or Thr54 IFABP. Caco-2 cells provided a model system for expression of Thr54 and Ala54 IFABP since these cells functionally mimick polarized small intestinal epithelial cells, but do not endogenously express IFABP. We produced permanently transfected Caco-2 cell lines expressing equivalent amounts of Ala54 and Thr54 IFABP and compared their ability to transport long-chain fatty acids from their apical to basolateral surfaces. Transfected cell monolayers, grown on filter supports, were apically exposed to ^{3}H-long-chain fatty acids for 5 min-48 hours and then the basolateral media was analyzed for ^{3}H lipid that had been transported across the monolayer. At all time points, more ^{3}H lipid was transported across the Thr54-expressing cells as compared to the Ala54-expressing cells. The observed difference in transport of long-chain fatty acids across Thr54-expressing cells as compared to Ala54-expressing cells appears to be specific for molecules that interact with IFABP, since no difference in ^{14}C-glucose transport was observed across these cells. The nature of the basolaterally secreted ^{3}H-lipids components was also analyzed and we found that Thr54-expressing cells secreted a 4-6 fold greater amount of triglyceride to the basolateral media than Ala54 cells. A comparison of the protein structures of Thr54 and Ala54 IFABP is being solved by 3-D NMR in collaboration with J. Sacchettini and J. Hamilton.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69046-04 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of the Glycogen Synthase Gene in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	M. Majer	Special Volunteer	CDNS, NIDDK
	D. Mott	Supervisory Res. Chemist	IHS

COOPERATING UNITS *(if any)*

Indian Health Service; Steno Diabetes Center, Denmark (O. Pedersen)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	.3	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Skeletal muscle glycogen synthase (encoded by GYS1 on chromosome 19q13.3) is the rate limiting enzyme in insulin-mediated non-oxidative glucose disposal. Our previous studies demonstrated an impairment of insulin-stimulated GYS1 activities in insulin resistant Pimas, and associations of NIDDM with the GYS1 locus were reported recently in Finish and Japanese populations. We have performed linkage and association analyses of GYS1 and seven additional DNA markers on 19q with NIDDM and with parameters of insulin action in the Pimas. We have found a significant association of NIDDM with GYS1 in the Pima Indians. We have performed a detailed comparative analysis of the GYS1 gene, mRNA, and protein product in insulin sensitive and insulin resistant Pimas. No mutations in GYS1 coding sequences were detected, neither did we find alterations of GYS1 mRNA expression, or of its basal enzymatic activity in insulin resistant Pimas. These results contrasted with a 25% reduction of immunoreactive GYS1 protein in insulin resistant subjects as detected by western blotting. Because no mutations were detected in DNA encoding this epitope, the difference in immunoreactivity may reflect post-translational modification(s) of the protein rather than a difference in the gene itself. We conclude that our data do not indicate alterations in the GYS1 gene as the cause for the observed association. It is possible that a different locus near GYS1 may be the contributing genetic element.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69051-03 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	K.M.V. Narayan	Visiting Scientist	DAES, NIDDK
	R.L. Hanson	Senior Staff Fellow	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	P.H. Bennett	Chief	PECRB, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; University of Pittsburgh.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prevention strategies will be developed to take advantage of the metabolic and behavioral risk factors for NIDDM which have been identified in research with the Pima Indians and elsewhere. Obesity, impaired glucose tolerance, hyperinsulinemia, physical inactivity, and high fat diet have been implicated as risk factors for NIDDM. Since these factors are potentially reversible with behavioral and pharmacologic therapy, strategies for prevention will be developed and tested. A feasibility study of diet and exercise treatment has been conducted among overweight Pima Indians with normal glucose tolerance. Knowledge and experience gained from this pilot study will be used in planning and implementing the national, multicenter clinical trial in prevention of NIDDM, in which the investigators will participate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69052-03 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of SH2-Domain PTPase by Insulin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Cantenberg Center for General & Tumor Immunology, The Hebrew University Jersusalem, Israel (D. Naor)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The abnormal regulation of insulin signalling in insulin-resistant subjects is associated with inadequate suppression of soluble protein tyrosine phosphatase (PTPase) activity in skeletal muscle. Multiple PTPases are likely to be involved in the signalling cascade. SH2-Domain PTPases are a class of PTPases selected as candidates for regulating tyrosine phosphorylation following insulin stimulation since it has been reported that such PTPases associate with several receptors and the post-receptor target, IRS1. SH2-Domain PTPase expression was detected in FAO cells, a rat insulin-sensitive hepatoma cell line, and in C2C12 cells, a mouse muscle cell line, using RT-PCR and degenerate primers. Sequence analysis of the amplified products revealed a high degree of identity with the hematopoietic SH2-Domain PTPase, PTP1C. We observed evidence of alternative splicing of the PTP1C pre-mRNA in human rhabdomyosarcoma cells and in EBV-transformed lymphocytes. A related PTPase PTP1D, (SH-PTP2, SYP) has been recently found to be expressed in adult liver and skeletal muscle. PTP1D pre-mRNA is alternatively spliced in a region corresponding to the catalytic domain of the PTPase. Relative amounts of the two PTP1D isotypes was not different in insulin resistant and sensitive subjects. This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69053-03 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Alternative Splicing by Insulin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alternative splicing is a powerful regulatory mechanism for generating subtle changes in isotype distribution. The insulin receptor itself is alternatively spliced in a developmental and tissue-specific manner. A change in distribution of the two isotypes has the potential to contribute to insulin resistance since the two isotypes possess different insulin responsive properties. Differing reports on changes in the insulin receptor isotype in diabetic and control subjects prompted us to examine the isotype distribution in Pima subjects and to characterize a potential role for insulin itself in the regulation of the alternative splicing of the insulin receptor. A small and significant difference in insulin receptor isotype was detected in insulin resistant Pima subjects compared to insulin sensitive Pima subjects using RT-PCR of RNA prepared from skeletal muscle biopsies. Insulin transiently influenced the insulin receptor isotype distribution following insulin treatment of FAO cells, a rat insulin-sensitive hepatoma cell line. These insulin inducible changes in insulin receptor isotype distribution are thought to occur as a consequence of hyperinsulinemic response to insulin resistance which has some other cause(s). This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69054-02 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energy Expenditure and Body Composition in Patients with Spinal Cord Injuries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Pratley	Senior Staff Fellow	CDNS, NIDDK
Others:	E. Ravussin	Visiting Scientist	CDNS, NIDDK
	M. Monroe	Pre-IRTA	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lean body mass is an important determinant of resting and 24-hour energy expenditure. Individuals who suffer spinal cord injuries experience a marked loss of muscle mass which may decrease energy expenditure and promote an increase in body fat. This project tests the hypothesis that energy expenditure is decreased in patients with spinal cord injuries as a consequence of their marked muscle atrophy. Resting metabolic rate will be measured by indirect calorimetry and body composition by dual-energy X-ray absorptiometry (DXA) in subjects with spinal cord injuries and compared to results obtained in age-matched able-bodied individuals. In addition, 24-hour energy expenditure will be measured in a respiratory chamber in a subgroup of paraplegic individuals who are fully independent in self care. A better understanding of the relationship between changes in body composition and energy expenditure in patients with spinal cord injuries may eventually suggest strategies to reduce the excess risk of diabetes and cardiovascular disease in this population. These studies also may improve our understanding of the contributions of muscle mass to energy expenditure in the general population as well as in other special populations characterized by a loss of muscle mass (such as the elderly).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69055-02 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Linkage of Markers on Chromosome 7 with NIDDM in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	B. Thompson	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Hospital for Sick Children, Toronto, Canada (Drs. S. Scherer and L.-C. Tsui)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes & Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.80	.80	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As insulin resistance and NIDDM may both have a genetic basis, we are searching systematically for the involved gene(s) using sib-pair linkage analyses of evenly spaced microsatellite markers in the Pima Indians of the Gila River Indian Community, a population with the highest known prevalence of this disease. We found evidence for linkage and association of insulin resistance and NIDDM with a cluster of microsatellite markers within a 2-3 cM genomic segment at the 7q21.3-q22.1 chromosomal band. In addition, marker D7S479, linked with NIDDM in the Pimas, is also linked with NIDDM in families of Northern European descent living in Utah. Based on these findings we conclude that there is a genetic element in the region 7q21.3-q22.1 contributing to diabetes susceptibility. Studies are in progress to identify this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69056-02 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Linkage Disequilbrium as a Method to Map Genes for Common, Complex Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS, NIDDK
Others:	S. Sell	Special Volunteer	CDNS, NIDDK
	L. Sandkuiji	Professor	University of Leiden The Netherlands

COOPERATING UNITS *(if any)*

Indian Health Service; Dept. of Neurogenetics, UCSF, San Francisco, Clinical Genetics (N. Freimer)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Dibaetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Identifying regions of maximal linkage disequilbrium between a disease phenotype and a particular marker allele is a valuable means to pinpoint the chromosomal location of the responsible gene. Indeed, most of the efforts to positionally clone human disease genes have relied on the measurement of linkage disequilbrium. Markers on chromosomes 4 and 21 will be used to carry out linkage disequilibrium analysis of the Pima genome. These chromosomes were chosen for this study because they have been extensively mapped genetically and physically, and gene poor and gene rich regions have been identified and correlated to sequence composition over extensive lengths of chromosome 21. We have identifed four additional chromosomal regions as possible linkage disequilibrium candidates because they show a high degree of genetic homogeneity in the Pima population. Increased homozygosity at these four microsatellite marker loci was found in the Pima genome following a random screen with 100 markers. This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69057-02 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Physical Mapping of various Phenotypic Traits in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Thompson	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.80	.80	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To produce a 25 cM base genetic map in Pima Indians, we have genotyped over 150 highly heterozygous short tandem repeat polymorphisms, STRPs, scattered across the genome in a data set of 1000 individuals comprising 250 nuclear families. The average heterozygosity in Pima Indians is approximately 11% lower than the reported in Caucasians for these STRPs. Linkage analysis using these STRP markers revealed regions on chromsomes 1, 6, and 7 that show linkage to various aspects of carbohydrate or fat metabolism or show linkage to diabetes. We have genotyped additional markers in the regions of linkage, and have started the physical mapping of a region on chromosome 1 covering approximately 9 cM with a number of YAC clones which include the original marker which showed linkage. A number of genes have been placed on the physical map including Phosphoglucomutase, Acyl Co-A Dehydrogenase, c-jun, Janus Kinase and Phosphodiesterase 4B. In addition, a number of unknown cDNAs also are located on the physical map. A region on chromosome 20 showed strong linkage to the quantitative character, stature. Analysis of additional markers supports the original linkage observation. A candidate gene, the bone morphogenetic protein 2 gene, BMP2, lies in this region. We screened this gene for structural variation. No consistent changes were observed. Sequence analysis of these individuals identified a single nucleotide substitution not detected with SSCP. This results in the substitution of a glycine for a valine, which was not associated with differences in stature. Previous analysis of the candidate gene GLUT2 on chromosome 3q showed linkage to percent body fat. The candidate gene Apolipoprotein-D had been linked to obesity in a British population. SSCP and sequence analysis of the Apolipoprotein gene did not support sib-pair linkage observation of linkage between obesity and chromosome 3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69058-02 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of PP-1 Components in Insulin Resistant Pimas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Prochazka	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	H. Mochizuki	Special Volunteer	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, University of Dundee, Scotland (P. Cohen); Hospital for Sick Children, Toronto, Canada (Dr. S. Scherer and Dr. L.-C. Tsui)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.4	.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin resistance is predictive of the development of non-insulin-dependent diabetes mellitus in Pima Indians, and this abnormality is associated with decreased rates of glycogen synthesis in skeletal muscle and of its regulatory enzyme complex, the glycogen-bound type protein phosphatase (PP1). Glycogen-associated PP1 activity is determined by an isoform of the catalytic subunit complexed with the glycogen-targeting regulatory (G) subunit, and structural alterations of either component could result in the biochemical abnormalities observed in insulin resistant Pimas. Three genetically distinct PP1 catalytic subunit isoforms are known (PP1 alpha, PP1 beta, and PP1 gamma). We have previously determined the exon-intron structure of the genes coding for PP1 alpha catalytic subunit (PPP1CA; 7 exons), PP1 beta (PPP1CB, 8 exons), PP1 gamma (PPP1CC, 7 exons), and PP1 regulatory G-subunit (PPP1R3, 4 exons), and we also positioned PPP1R3 on chromosome 7q. We have isolated polymorphic repeat markers at all PPP1 genes, and so far we have analyzed markers at PPP1CA and PPP1CB. We found no evidence for linkage of PPP1CA or PPP1CB with fasting insulin or insulin action in vivo and similar linkage analyses of repeat markers at PPP1CC and PPP1R3 are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69059-01 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energy Metabolism in Patients with Huntington's Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Pratley	Senior Staff Fellow	CDNS, NIDDK
Others:	E. Ravussin	Visiting Scientist	CDNS, NIDDK
	J. Caviness	Neurologist	Mayo Clinic, Scottsdale

COOPERATING UNITS *(if any)*

Indian Health Service; Mayo Clinic, Scottsdale, Arizona.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Huntington's Disease is an autosomal dominant neurologic disorder characterized by chorea, eye movement abnormalities, and dementia. Significant weight loss is a common finding early in the course of the disease despite reportedly normal or increased caloric intakes. The cause of this apparent increase in energy expenditure is not known. We hypothesize that energy expenditure is increased in patients with Huntigton's Disease as a consequence of the movement disorder. To test this, basal metabolic rate, 24-hour energy expenditure, and non-resting energy expenditure (all measured using the human respiratory chamber on the Research Ward of the Clinical Diabetes and Nutrition Section), and 7-day free-living energy expenditure (measured using the doubly-labeled water technique) will be compared in three groups of patients with Huntington's Disease with varying degrees of chorea (asymptomatic, mild, and moderate) and age, sex, and weight matched controls. To correct energy expenditure for differences in metabolically active (primarily lean) body mass among subjects, all individuals will have body composition measured by anthropometry, bioelectrical impedance analysis, and dual energy X-ray absorptiometry scanning. In addition, since the reported prevalence of non-insulin dependent diabetes is 7 times higher in patients with Huntington's Disease than in the general population, all patients will also undergo an oral glucose tolerance test with measurement of plasma insulin levels. Comparison of asymptomatic patients with Huntington's Disease and controls will address whether an inherent abnormality in energy metabolism is present in Huntington's Disease, whereas comparison of patients with varying degrees of chorea will address the contribution of the movement disorder to the apparent increase in energy expenditure. In addition to providing insight into the pathophysiologic abnormalities in Huntington's Disease, this study may also improve the care of patients with Huntington's Disease by providing more precise estimates of their caloric needs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69060-01 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Fatty Acid Absorption and Metabolism in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Pratley	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; University of Southampton (Dr. S. Wooton and Dr. J. Murphy)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.15	.15	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The intestinal fatty acid binding protein (IFABP) is present in humans in two forms which differ by a alanine to threonine substitution at amino acid 54. *In vitro*, the threonine form (Thr54) is characterized by a two-fold higher binding affinity for long chain fatty acids than the alanine form (Ala54). The Thr54 form also is associated with higher plasma insulin concentrations and insulin resistance in the Pima Indians. This project examines whether the *in vitro* binding differences are physiologically significant. Net dietary fatty acid absorption, the kinetics of appearance of dietary fatty acids into plasma, and the time course of lipid oxidation will be determined following a standard test meal incorporating [1-^{13}C]-palmitic acid as a tracer of fatty acid metabolism in healthy subjects homozygous for either the Thr54 form (n=20) or the Ala54 form of IFABP (n=20). These results will be related to rates of 24-hour substrate oxidation (measured in the respiratory chamber).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69061-01 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Primary Skeletal Muscle Tissue Culture from Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Thompson	Senior Staff Fellow	CDNS, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.15	.15	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary skeletal muscle cell lines have been established from muscle biopsies from Pima Indians with varying in vivo insulin sensitivities. Muscle biopsies were performed in conjunction with a hyperinsulinemic euglycemic clamp to measure in vivo insulin sensitivity. The cells derived from these biopsies maintained the ability to fuse and the resulting myotubes morphologically resemble myocytes. Antibodies directed against α-actinin and myosin detect both of these proteins in fixed cells indicating cultures contain cells of muscle lineage. Muscle-specific creatine kinase is also expressed by these cells. RNA encoding GLUT4, the skeletal muscle glucose transporter was detected as was RNA encoding MYOD, MYF5, and MYOGENNIN muscle-specific differentiation factors. The myoblasts also retain differing capacities for non-oxidative glucose metabolism. Glycogen is produced in these cells in a time dependent manner. Glycogen production also increases in response to increasing concentrations of insulin. Glycogen production was also measured, in these cell lines, in response to insulin-stimulation. The variation in glycogen production between cell lines correlated well with in vivo measures of insulin resistance as measured by the hyperinsulinemic euglycemic clamp indicating the retention of insulin resistance in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69062-01 PECR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renoprotection in Non-Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.G. Nelson	Special Expert	PECRB, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

Cleveland Clinic Foundation; Stanford University; Indian Health Service

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Phoenix Epidemiology and Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDDK, Phoenix, Arizona

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The functional characteristics of the renal glomerulus have been investigated in Pima Indians of the Gila River Indian Community to identify the underlying pathogenetic mechanisms involved in the initiation and progression of renal disease in non-insulin-dependent diabetes mellitus (NIDDM).

A randomized, double-blinded, placebo-controlled clinical trial will be performed in diabetic Pima Indians with normal urinary albumin excretion or early microalbuminuria to test the hypothesis that blockade of the renin-angiotensin system can prevent or attenuate the development and progression of early diabetic nephropathy (RPF) and fractional clearances of albumin and IgT will be made. Masked treatment with drug or placebo will be converted to active treatment in those who develop macroalbuminuria (albumin-to-creatinine ratio ≥300 mg/g), but both the patient and the investigators will remain blinded to the previous treatment. Kidney biopsies will be performed after five years in 30 subjects who initially had normoalbuminuria and will be selected equally from the drug and placebo groups. Morphometric analysis will be used to determine differences in the prevalence of global sclerosis, the breadth of epithelial foot processes, and the fractional mesangial volume between treatment groups.

The major outcome measure will be renal insufficiency and subjects will be followed to this endpoint. Other measures of renoprotection that will be assessed are 1) change in albumin excretion, 2) change in GFR, and 3) glomerular morphology. This project, in part, represents an extension of work previously reported as Project Number Z01 DK 69037.

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